
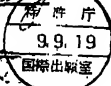


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This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above. By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS MAR 17 1997 Carolyn S. Shaw					



PATENT APPLICATION SERIAL NO. \_\_\_\_\_

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Priscilla Tarleton

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PROVISIONAL PATENT APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION for patent under 37 CFR 1.53 (b)(2).

LAST NAME	REVOLUTIONARY/APPLICANT(S) FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
1-00 2-00 3-11 Pacella Volrath Mard	Maria Sandra Eric	A. L. E.	Raleigh, North Carolina Durham, North Carolina Durham, North Carolina

(TITLE OF THE INVENTION (DO NOT EXCEED 500 CHARACTERS))

Promoters from Plant Protoporphyrinogen Oxidase Genes

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ENCLOSED APPLICATION PARTS (check all that apply)

<input checked="" type="checkbox"/> 55 pages of Specification (and any claims)	<input checked="" type="checkbox"/> 1 page of Abstract (page 55)
<input type="checkbox"/> sheets of Drawing(s)	<input type="checkbox"/> Other (specify)

METHOD OF PAYMENT

The Commissioner is hereby authorized to charge filing fees and additional fees required to Disposal Account number 87-4985	PROVISIONAL FILING FEE AMOUNT: \$130.00
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☐ U.S. Government agency and contract number \_\_\_\_\_ (If the invention was made by an agency of the United States Government or under a contract with an agency of the United States Government)

Respectfully submitted,

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60,020003

# PROMOTERS FROM PLANT PROTOPORPHYRINOGEN OXIDASE GENES

## CROSS-REFERENCE TO RELATED PROVISIONAL

This provisional application is related to U.S. provisional application serial no.

5 60/013,612 filed February 28, 1996.

## FIELD OF THE INVENTION

This invention relates to novel DNA sequences which function as promoters of transcription of associated DNA sequences in plants. More specifically, this invention relates to  
10 novel promoters which are naturally associated with plant protoporphyrinogen oxidase (protoph)  
coding sequences.

## BACKGROUND OF THE INVENTION

1. The Protoph Enzyme and its Involvement in the Chlorophyll/Heme Biosynthetic  
15 Pathway

The biosynthetic pathways which lead to the production of chlorophyll and heme share a number of common steps. Chlorophyll is a light harvesting pigment present in all green photosynthetic organisms. Heme is a cofactor of hemoglobin, cytochromes, P450 mixed-function oxygenases, peroxidases, and catalases (see, e.g. Lehninger, *Biochemistry*, Worth Publishers,  
20 New York (1975)), and is therefore a necessary component for all aerobic organisms.

The last common step in chlorophyll and heme biosynthesis is the oxidation of protoporphyrinogen IX to protoporphyrin IX. Protoporphyrinogen oxidase (referred to herein as "protoph") is the enzyme which catalyzes this last oxidation step (Matringe *et al.*, *Biochem. J.* 260: 231 (1989)).

25 The protoph enzyme has been purified either partially or completely from a number of organisms including the yeast *Saccharomyces cerevisiae* (Labbe-Bois and Labbe, In *Biosynthesis of Heme and Chlorophyll*, E.H. Dailey, ed. McGraw Hill: New York, pp. 235-285 (1990)), barley etioplasts (Jacobs and Jacobs, *Biochem. J.* 244: 219 (1987)), and mouse liver (Dailey and Karr, *Biochem. J.* 269: 2697 (1987)). Genes encoding protoph have been isolated from two prokaryotic

organisms, *Escherichia coli* (Sasamar *et al.*, *Can. J. Microbiol.* 39: 1155 (1993)) and *Bacillus subtilis* (Dailey *et al.*, *J. Biol. Chem.* 269: 813 (1994)). These genes share no sequence similarity; neither do their predicted protein products share any amino acid sequence identity. The *E. coli* protein is approximately 21 kDa, and associates with the cell membrane. The *B. subtilis* protein is 51 kDa, and is a soluble, cytoplasmic activity.

Protox encoding cDNAs have now also been isolated from humans (see Nishimura *et al.*, *J. Biol. Chem.* 270(14): 8076-8080 (1995) and plants (International application no. PCT/JP95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659).

## II. The Protox Gene as a Herbicide Target

The use of herbicides to control undesirable vegetation such as weeds or plants in crops has become almost a universal practice. The relevant market exceeds a billion dollars annually. Despite this extensive use, weed control remains a significant and costly problem for farmers.

Effective use of herbicides requires sound management. For instance, time and method of application and stage of weed plant development are critical to getting good weed control with herbicides. Since various weed species are resistant to herbicides, the production of effective herbicides becomes increasingly important.

Unfortunately, herbicides that exhibit greater potency, broader weed spectrum and more rapid degradation in soil can also have greater crop phytotoxicity. One solution applied to this problem has been to develop crops which are resistant or tolerant to herbicides. Crop hybrids or varieties resistant to the herbicides allow for the use of the herbicides without attendant risk of damage to the crop. Development of resistance can allow application of a herbicide to a crop where its use was previously precluded or limited (e.g. to pre-emergence use) due to sensitivity of the crop to the herbicide. For example, U.S. Patent No. 4,761,373 to Anderson *et al.* is directed to plants resistant to various imidazolinone or sulfonamide herbicides. The resistance is conferred by an altered acetohydroxyacid synthase (AHAS) enzyme. U.S. Patent No. 4,975,374 to Gundman *et al.* relates to plant cells and plants containing a gene encoding a mutant glutamine synthetase (GS) resistant to inhibition by herbicides that were known to inhibit GS, e.g.

phosphinothricin and methanone sulfoximine. U.S. Patent No. 5,013,659 to Bedford *et al.* is directed to plants that express a mutant acetolactate synthase which renders the plants resistant to inhibition by sulfonylurea herbicides. U.S. Patent No. 5,162,602 to Somers *et al.* discloses plants tolerant to inhibition by cyclohexanedione and aryloxyphenoxypropionic acid herbicides. The tolerance is conferred by an altered acetyl coenzyme A carboxylase (ACCase).

The protox enzyme serves as the target for a variety of herbicidal compounds. The herbicides that inhibit protox include many different structural classes of molecules (Duke *et al.*, *Weed Sci.* 39: 465 (1991); Nandihalli *et al.*, *Pesticide Biochem. Physiol.* 43: 193 (1992); Matringe *et al.*, *FEBS Lett.* 245: 35 (1989); Yanase and Andoh, *Pesticide Biochem. Physiol.* 35: 70 (1989)). These herbicidal compounds include the diphenylethers [e.g. acifluorfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid; its methyl ester; or oxyfluorfen, 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluorobenzene)], oxidiazoles, (e.g. oxidiazon, 3-[2,4-dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2-(3H)-one, cyclic imides (e.g. S-23142, *N*-(4-chloro-2-fluoro-5-propargyloxyphenyl)-3,4,5,6-tetrahydrophthalimide; chlorophthalim, *N*-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide), phenyl pyrazoles (e.g. TNPP-ethyl, ethyl 2-[1-(2,3,4-trichlorophenyl)-4-nitropyrzazolyl-5-oxy]propionate; M&B 39279), pyridine derivatives (e.g. LS 82-556), and phenopylate and its *O*-phenylpyrrolidino- and piperidinocarbonate analogs. Many of these compounds competitively inhibit the normal reaction catalyzed by the enzyme, apparently acting as substrate analogs.

Typically, the inhibitory effect on protox is determined by measuring fluorescence at about 622 to 635 nM, after excitation at about 395 to 410 nM (see, e.g. Jacobs and Jacobs, *Enzyme* 28: 206 (1982); Sherman *et al.*, *Plant Physiol.* 97: 280 (1991)). This assay is based on the fact that protoporphyrin IX is a fluorescent pigment, and protoporphyrinogen IX is nonfluorescent.

The predicted mode of action of protox-inhibiting herbicides involves the accumulation of protoporphyrinogen IX in the chloroplast. This accumulation is thought to lead to leakage of protoporphyrinogen IX into the cytosol where it is oxidized by a peroxidase activity to protoporphyrin IX. When exposed to light, protoporphyrin IX can cause formation of singlet oxygen in the cytosol. This singlet oxygen can in turn lead to the formation of other reactive

oxygen species, which can cause lipid peroxidation and membrane disruption leading to rapid cell death (Lue *et al.*, *Plant Physiol.* 102: 881 (1993)).

Not all protox enzymes are sensitive to herbicides which inhibit plant protox enzymes.

- Both of the protox enzymes encoded by genes isolated from *Escherichia coli* (Sassarman *et al.*, *Can. J. Microbiol.* 39: 1155 (1993)) and *Bacillus subtilis* (Dailey *et al.*, *J. Biol. Chem.* 269: 813 (1994)) are resistant to these herbicidal inhibitors. In addition, mutants of the unicellular alga *Chlamydomonas reinhardtii* resistant to the phenylimide herbicide S-23142 have been reported (Kataoka *et al.*, *J. Pesticide Sci.* 15: 449 (1990); Shibata *et al.*, In *Research in Photosynthesis*, Vol. III, N. Murata, ed. Kluwer/Netherlands, pp. 567-570 (1992)). At least one of these mutants appears to have an altered protox activity that is resistant not only to the herbicidal inhibitor on which the mutant was selected, but also to other classes of protox inhibitors (Oshio *et al.*, *Z. Naturforsch.* 48c: 339 (1993); Sato *et al.*, In *ACS Symposium on Porphyrin Pesticides*, S. Duke, ed. ACS Press: Washington, D.C. (1994)). A mutant tobacco cell line has also been reported that is resistant to the inhibitor S-21432 (Che *et al.*, *Z. Naturforsch.* 48c: 350 (1993). In addition, modified, inhibitor-resistant forms of plant protox coding sequences have been described in international application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659.

### 20 III. Regulation of Protox Gene Expression

The bulk of the research related to the protox gene which has been conducted thus far has focused upon the coding sequence and modifications to this enzyme which may render it resistant to protox inhibitors. No information is available in the art with regard to the regulatory elements which control and promote the expression of protox coding sequences in plants.

# SUMMARY OF THE INVENTION

The present invention is based on the discovery that the promoter regions naturally associated with the plant protoporphyrinogen oxidase (protoph) coding sequences, referred to herein generally as the "protoph promoter", are useful for promoting expression of a heterologous coding sequence in a plant.

In accordance with this discovery, the present invention provides an isolated DNA molecule comprising a plant protoph promoter. The present invention further provides a chimeric gene comprising a plant protoph promoter operably linked to a heterologous coding sequence. Plant tissue and plants containing such a chimeric gene are also provided.

In one aspect of the invention the protoph promoter is used to express herbicide resistant forms of herbicide target proteins in a plant to confer tolerance to the herbicide. According to this aspect, the protoph promoter may be operably linked to a coding sequence for a herbicide-resistant plant protoph protein which is resistant to inhibitors of unmodified plant protoph protein.



# DESCRIPTION OF THE SEQUENCE LISTING

- SEQ ID No. 1: DNA coding sequence for an *Arabidopsis thaliana* protox-1 protein.
- 5 SEQ ID No. 2: *Arabidopsis thaliana* protox-1 amino acid sequence encoded by SEQ ID No. 1.
- SEQ ID No. 3: DNA coding sequence for an *Arabidopsis thaliana* protox-2 protein.
- 10 SEQ ID No. 4: *Arabidopsis thaliana* protox-2 amino acid sequence encoded by SEQ ID No. 3
- SEQ ID No. 5: DNA coding sequence for a maize protox-1 protein.
- SEQ ID No. 6: Maize protox-1 amino acid sequence encoded by SEQ ID No. 5
- SEQ ID No. 7: DNA coding sequence for a maize protox-2 protein.
- 15 SEQ ID No. 8: Maize protox-2 amino acid sequence encoded by SEQ ID No. 7
- SEQ ID No. 9: DNA coding sequence for a wheat protox-1 protein.
- SEQ ID No. 10: Wheat protox-1 amino acid sequence encoded by SEQ ID No. 9.
- SEQ ID No. 11: DNA coding sequence for a soybean protox-1 protein.
- SEQ ID No. 12: Soybean protox-1 protein encoded by SEQ ID No. 11.
- 20 SEQ ID NO. 13: Promoter sequence from *Arabidopsis thaliana* protox-1 gene.
- SEQ ID NO. 14: Promoter sequence from *Zea mays* (maize) protox-1 gene.

## DEFINITIONS

As used herein a "plant protox promoter" is used to refer to the regulatory region which naturally occurs immediately upstream of a protoporphyrinogen oxidase (protox) coding sequence in a plant and is responsible, in its naturally occurring state, for regulating the transcription of the associated protox coding sequence. The plant protox promoter includes the DNA region directly involved in binding of RNA polymerase to initiate transcription and additional upstream regulatory cis-elements which influence the transcription of an operably linked coding sequence.

As used herein a "gene" is used to refer to a DNA molecule which includes (1) a coding sequence and (2) associated regulatory regions which promote and regulate the transcription of the coding sequence in a suitable host cell. The coding sequence may encode a useful transcript (e.g. antisense RNA) or polypeptide produced by translation of the encoded transcript. A gene includes at a minimum, in 5'-3' orientation, a promoter region, a coding sequence and a transcription terminator. A gene may also include additional regulatory regions which can occur as part of the minimal elements (e.g. leaders or signal peptides within the coding sequence) or as discrete elements (e.g. introns).

As used herein a "chimeric gene" refers to a gene which does not naturally occur wherein at least one component part is heterologous with respect to another component part. As used herein to describe the present invention a "chimeric gene" refers to a gene which includes the promoter of the invention operably linked to a heterologous coding sequence.

As used herein with reference to the relationship between a promoter and a coding sequence, the term "heterologous" is used to refer to a relationship which does not naturally occur. For instance, a coding sequence is considered heterologous with respect to a promoter sequence if it is different from the coding sequence that naturally occurs in association with the promoter sequence. This includes modified forms of coding sequences which are naturally associated with a subject promoter. Accordingly, a modified, inhibitor-resistant protox coding sequence is considered to be heterologous with respect to the promoter that is naturally associated with the unmodified, inhibitor-sensitive form of this coding sequence.

As used herein, the term "substantial sequence homology" is used to indicate that a nucleotide sequence (in the case of DNA or RNA) or an amino acid sequence (in the case of a protein or polypeptide) exhibits substantial structural and functional equivalence with another nucleotide or amino acid sequence. Any functional or structural differences between sequences having substantial sequence homology will be de minimis; that is they will not affect the ability of the sequence to function as indicated in the present application. For example, a sequence which has substantial sequence homology with a DNA sequence disclosed to be a plant protox promoter will be able to direct the same level and pattern of expression of an associated DNA sequence as the plant protox promoter. Sequences that have substantial sequence homology with the sequences disclosed herein are usually variants of the disclosed sequence, such as mutations, but may also be synthetic sequences. Structural differences are considered de minimis if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics. Such characteristics can include, for example, immunological reactivity, enzyme activity, structural protein integrity, etc.

Two nucleotide sequences may have substantial sequence homology if the sequences have at least 70 percent, more preferably 80 percent and most preferably 90 percent sequence similarity between them. Two amino acid sequences have substantial sequence homology if they have at least 50 percent, preferably 70 percent, and most preferably 90 percent similarity between the active portions of the polypeptides. In the case of promoter DNA sequences, "substantial sequence homology" also refers to those fragments of a promoter DNA sequence that are able to operate to promote the expression of associated DNA sequences. Such operable fragments of a promoter DNA sequence may be derived from the promoter DNA sequence, for example, by cleaving the promoter DNA sequence using restriction enzymes, synthesizing in accordance with the sequence of the promoter DNA sequence, or may be obtained through the use of PCR technology. Mullis et al., *Meth. Enzymol.*, 155:335-350 (1987); Erlich (ed.), *PCR Technology*, Stockton Press (New York 1989).

A promoter DNA sequence is said to be "operably linked" to a second DNA sequence if the two are situated such that the promoter DNA sequence influences the transcription or translation of the second DNA sequence. For example, if the second DNA sequence codes for

the production of a protein, the promoter DNA sequence would be operably linked to the second DNA sequence if the promoter DNA sequence affects the expression of the protein product from the second DNA sequence. For example, in a DNA sequence comprising a promoter DNA sequence physically attached to a coding DNA sequence in the same chimeric construct, the two sequences are likely to be operably linked.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to promoter DNA sequences which are naturally associated with coding sequences for plant protoporphyrinogen oxidase (referred to herein as "protox"; see international application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659 and co-pending provisional application entitled "DNA Molecules Encoding Plant Protoporphyrinogen Oxidase and Inhibitor Resistant Mutants Thereof" filed on the same day as the instant application). These protox promoter sequences have been found to be useful for the expression of a heterologous coding sequence in a plant.

The promoter sequence for the *Arabidopsis thaliana* protox-1 coding sequence (SEQ ID No. 1) is provided as SEQ ID No. 13. Isolation of this promoter from a genomic library using the associated coding sequence as a probe is described in Example 1. The promoter sequence for the maize protox-1 coding sequence (SEQ ID No. 5) is provided as SEQ ID No. 14. Isolation of this promoter from a genomic library using the associated coding sequence as a probe is described in Example 4.

The approach used to isolate the *Arabidopsis* and maize protox-1 promoters can be used to isolate the promoter sequence from any plant protox gene. Any protox coding sequence which shares sufficient homology to hybridize to the protox coding sequence associated with the promoter of interest may be used as a probe in this approach. Since the respective protox-1 and protox-2 coding sequences from all plants are contemplated to share this requisite degree of homology, the choice of which protox coding sequence is used as a probe is not considered critical. However, for optimal hybridization results it is preferable to use the most closely related protox coding sequence. Most preferably, the coding sequence used as a probe is from the same

plant species as the protox promoter of interest and is the coding sequence naturally associated with the promoter.

The plant protox promoter of the present invention includes the *Arabidopsis* protox-1 promoter sequence set forth in SEQ Id No. 13 as well as corresponding protox-1 promoter sequences available from other plant species as indicated above. The present invention also includes functional fragments of these DNA sequences which retain the ability to regulate expression of an operably linked coding sequence in the same manner as the exemplified protox promoter sequence. Such functional fragments may be identified through deletion analyses or other standard techniques used in the art to identify protox promoter activity (see, e.g. pages 546-549 of "Genes IV", ed. by Lewin, Oxford Univ. Press (1990)). The present invention also includes DNA sequences having substantial sequence homology with the protox promoters available from plant genes which confer an equivalent level and pattern of expression upon an operably linked sequence. Such promoter sequences may be obtained through modification of the protox promoters isolated from plant genes and are considered functionally equivalent derivatives of the plant protox promoters.

As illustrated in the examples below, the DNA sequences, vectors and transgenic plants of the present invention comprise a promoter sequence derived from a plant protox gene. The protox promoter DNA sequences are preferably linked operably to a coding DNA sequence, for example a DNA sequence which is transcribed into a useful RNA transcript such as an antisense transcript, or a coding sequence which is ultimately expressed in the production of a useful protein product.

In a preferred embodiment, the protox promoter is used to direct the expression of a modified herbicide target enzyme which is resistant to herbicides at levels that inhibit the corresponding unmodified version of the enzyme. Such modified herbicide-resistant enzymes include herbicide-resistant forms of imidazoglycyl phosphate dehydratase (IGPD; see WO 9426909 published Nov. 24, 1994), EPSP synthase (see U.S. Pat. Nos. 4,535,060; 4,769,061; 4,940,835 and EP 550,633), glutamine synthetase (GS; see U.S. Patent No. 4,975,374), acetyl coenzyme A carboxylase (ACCase; see U.S. Patent No. 5,162,602), and acetolactate synthase (see U.S. Patent Nos. 4,761,373; 5,304,732; 5,331,107; 5,013,659; 5,141,870; and 5,378,824).

In a most preferred embodiment, the protox promoter is used to direct the expression of a modified protox enzyme which is resistant to protox inhibitors as illustrated in Examples 2-3 (see also International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659 whose relevant parts are herein incorporated by reference; see also co-pending application entitled "DNA Molecules Encoding Plant Protoporphyrinogen Oxidase and Inhibitor Resistant Mutants Thereof" filed on the same day as the instant application).

The transgenic plants of the present invention may be transformed by any method of transformation known in the art. These methods include, for instance, transformation by direct infection or co-cultivation of plants, plant tissue or cells with *Agrobacterium tumefaciens*; Horsch *et al.*, *Science*, 225: 1229 (1985); Marton, "Cell Culture and Somatic Cell Genetic of Plants", vol 1, pp 514-521 (1984); direct gene transfer into protoplasts; Paszkowski *et al.*, *EMBO J.* 12: 2717 (1984); Loerz *et al.*, *Mol. Gen. & Genet.* 1199:178 (1985); Fromm *et al.*, *Nature* 319:719 (1986); microprojectile bombardment, Klein *et al.*, *BioTechnology*, 6:559-563 (1988); injection into protoplasts cultured cells and tissues, Reich *et al.*, *BioTechnology*, 4:1001-1004 (1986); or injection into meristematic tissues of seedlings and plants as described by De La Pena *et al.*, *Nature*, 325:274-276 (1987); Hooymaas-Van Slooteren *et al.*, *Nature*, 311:763-764 (1984); Grimley *et al.*, *BioTechnology*, 6:185 (1988); and Grimley *et al.*, *Nature*, 325:177 (1988).

The invention is illustrated in more detail by the following examples, without implying any restriction to what is described therein.

## EXAMPLES

EXAMPLE 1: Isolation of the *Arabidopsis thaliana* Protox-1 promoter sequence

- A Lambda Zap II genomic DNA library prepared from *Arabidopsis thaliana* (Columbia, whole plant) was purchased from Stratagene. Approximately 125,000 phage were plated at a density of 25,000 pfu (plaque forming units) per 15 cm Petri dish and duplicate lifts were made onto Colony/Plaque Screen membranes (NEN Dupont). The plaque lifts were probed with the *Arabidopsis* Protox-1 cDNA (SEQ ID No. 1 labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization and wash conditions were at 65°C as described in Church and Gilbert, *Proc. Natl. Acad. Sci. USA* 81: 1991-1995 (1984). Positively hybridizing plaques were purified and *in vivo* excised into pBluescript plasmids. Sequence from the genomic DNA inserts was determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). One clone, AraPT1Pro, was determined to contain 580 bp of *Arabidopsis* sequence upstream from the initiating methionine (ATG) of the Protox-1 protein coding sequence. This clone also contains coding sequence and introns that extend to bp 1241 of the Protox-1 cDNA sequence. The 580 bp 5' noncoding fragment is the putative *Arabidopsis* Protox-1 promoter, and the sequence is set forth in SEQ ID No. 13.

AraPT1Pro was deposited December 14, 1995, as pWDC-11 (NRRL #B-21515)

EXAMPLE 2: Construction of plant transformation vectors expressing altered Protox-1 genes behind the native *Arabidopsis* Protox-1 promoter

- A full-length cDNA of the appropriate altered *Arabidopsis* Protox-1 cDNA is isolated as an EcoRI-XhoI partial digest fragment and cloned into the plant expression vector pCGN1761ENX (see Example 9 of International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659). This plasmid is digested with NcoI and BamHI to produce a fragment comprised of the complete Protox-1 cDNA plus a transcription terminator from the 3' untranslated sequence of the *trnI* gene of *Agrobacterium tumefaciens*. The AraPT1Pro plasmid described above is digested with NcoI and BamHI to produce a fragment comprised of pBluescript and the 580 bp putative *Arabidopsis* Protox-1 promoter. Ligation of these two fragments produces a fusion of the altered protox cDNA to the native protox promoter.

The expression cassette containing the Protopx-1 promoter/Protopx-1 cDNA/trnI terminator fusion is excised by digestion with KpnI and cloned into the binary vector pCIB200. The binary plasmid is transformed by electroporation into *Agrobacterium* and then into *Arabidopsis* using the vacuum infiltration method (Bechtold *et al.* *C.R. Acad. Sci. Paris* 316: 1194-1199 (1993)).

- 5 Transformants expressing altered protox genes are selected on kanamycin or on various concentrations of protox inhibiting herbicide.

10 **EXAMPLE 3: Production of herbicide tolerant plants by expression of a native Protopx-1 promoter/alterred Protopx-1 fusion**

Using the procedure described above, an *Arabidopsis* Protopx-1 cDNA containing a TAC to ATG (Tyrosine to Methionine) change at nucleotides 1306-1308 in the Protopx-1 sequence (SEQ ID No.1) was fused to the native Protopx-1 promoter fragment and transformed into

15 *Arabidopsis thaliana*. This altered Protopx-1 enzyme (AraC-2Met) has been shown to be >10fold more tolerant to various protox-inhibiting herbicides than the naturally occurring enzyme when tested in a bacterial expression system (see Example 5 of copending U.S. application entitled "DNA Molecules Encoding Plant Protoporphyrinogen Oxidase and Inhibitor Resistant Mutants Thereof" filed on the same day as the instant application). Seed from the vacuum infiltrated plants

20 was collected and plated on a range (10.0nM-1.0uM) of a protox inhibitory aryluracil herbicide of formula XVII. Multiple experiments with wild type *Arabidopsis* have shown that a 10.0nM concentration of this compound is sufficient to prevent normal seedling germination. Transgenic seeds expressing the AraC-2Met altered enzyme fused to the native Protopx-1 promoter produced

25 normal *Arabidopsis* seedlings at herbicide concentrations up to 500nM, indicating at least 50-fold higher herbicide tolerance when compared to wild-type *Arabidopsis*. This promoter/alterred protox enzyme fusion therefore functions as an effective selectable marker for plant transformation. Several of the plants that germinated on 100.0nM of protox-inhibiting herbicide were transplanted to soil, grown 2-3 weeks, and tested in a spray assay with various concentrations of the protox-inhibiting herbicide. When compared to empty vector control

30 transformants, the AraPT1Pro/AraC-2Met transgenics were >10fold more tolerant to the herbicide spray.



**EXAMPLE 4: Isolation of a Maize Protoplast-1 promoter sequence**

- A Zea Mays (Missouri 17 inbred, et al. inbred seedlings) genomic DNA library in the Lambda FIX II vector was purchased from Stratagene. Approximately 250,000 pfu of the library was plated at a density of 50,000 phage per 15 cm plate and duplicate lifts were made onto Colony/Plaque screen membranes (NEN Dupont). The plaque lifts were probed with the maize Protoplast-1 cDNA labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization and wash conditions were at 65°C as described in Church and Gilbert, *Proc. Natl. Acad. Sci. USA* 81: 1991-1995 (1984). Lambda phage DNA was isolated from three positively hybridizing phage using the Wizard Lambda Preps DNA Purification System (Promega).
- Analysis by restriction digest, hybridization patterns, and DNA sequence analysis identified a lambda clone containing approximately 3.5 kb of maize genomic DNA located 5' to the maize Protoplast-1 coding sequence previously isolated as a cDNA clone. This fragment is contemplated to include the maize Protoplast-1 promoter. The sequence of this fragment is set forth in SEQ ID NO 14. From nucleotide 1 to 3532, this sequence is comprised of 5' noncoding sequence. From nucleotide 3533 to 3848, this sequence encodes the 5' end of the maize Protoplast-1 protein.

A plasmid containing the sequence of SEQ ID NO. 14 fused to the remainder of the maize Protoplast-1 coding sequence was deposited March 19, 1996 as pWDC-14 (NRRL #B-21546).

**EXAMPLE 5: Construction of Plant Transformation Vectors**

- Numerous transformation vectors are available for plant transformation, and the promoters and chimeric genes of this invention can be used in conjunction with any such vectors. The selection of vector for use will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *neptII* gene which confers resistance to kanamycin and related antibiotics (Messing & Vieira, *Gene* 19: 259-268 (1982); Bevan *et al.*, *Nature* 304: 184-187 (1983)), the *bar* gene which confers resistance to the herbicide phosphinothricin (White *et al.*, *Nucl Acids Res* 18: 1062 (1990), Spencer *et al.* *Theor Appl Genet* 79: 625-631 (1990)), the *aph* gene which confers resistance to

the antibiotic hygromycin (Blochinger & Diggelmann, *Mol Cell Biol* 4: 2929-2931), and the *dhfr* gene, which confers resistance to methotrexate (Bourouis *et al.*, *EMBO J.* 2(7): 1099-1104 (1983)).

#### 5. (1) Construction of Vector Suitable for *Agrobacterium* Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, *Nucl. Acids Res.* (1984)) and pXYZ. Below the construction of two typical vectors is described.

#### 10 Construction of pCIB200 and pCIB2001

The binary vectors pCIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and was constructed in the following manner. pTJS75kan was created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, *J. Bacteriol.* 164: 446-455 (1985)) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Messing & Vieira, *Gene* 19: 259-268 (1982); Bevan *et al.*, *Nature* 304: 184-187 (1983); McBride *et al.*, *Plant Molecular Biology* 14: 266-276 (1990)). *XhoI* linkers were ligated to the *EcoRV* fragment of pCIB7 which contains the left and right T-DNA borders, a plant selectable *noshptII* chimeric gene and the pUC polylinker (Rothstein *et al.*, *Gene* 53: 153-161 (1987)), and the *XhoI*-digested fragment was cloned into *Sall*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19 (1338)). pCIB200 contains the following unique polylinker restriction sites: *EcoRI*, *SstI*, *KpnI*, *BglIII*, *XbaI*, and *Sall*. pCIB2001 is a derivative of pCIB200 which created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI*, *SstI*, *KpnI*, *BglIII*, *XbaI*, *Sall*, *MluI*, *BclI*, *AvrII*, *PstI*, *HpaI*, and *SnaI*. pCIB2001, in addition to containing these unique restriction sites also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function

for mobilization between *E. coli* and other hosts, and the *oriT* and *oriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

5 Construction of pCIB10 and Hygromycin Selection Derivatives thereof

The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants, T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium*. Its construction is described by Rothstein *et al.*, *Gene* 53: 153-161 (1987). Various derivatives of pCIB10 have been constructed which incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al.*, *Gene* 25: 179-188 (1983). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

(2) Construction of Vectors Suitable for non-*Agrobacterium* Transformation.

15 Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques which do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (e.g. PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Below, the construction of some typical vectors is described.

Construction of pCIB3064

23 pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide basta (or phosphinothricin). The plasmid pCIB246

comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites were mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *SspI* and *PvuII*. The new restriction sites were 96 and 37 bp away from the unique *Sall* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 was designated pCIB3025. The GUS gene was then excised from pCIB3025 by digestion with *Sall* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 was obtained from the John Innes Centre, Norwich and the 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* was excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.* EMBO J 6: 2519-2523 (1987)). This generated pCIB3064 which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI*, *PstI*, *HindIII*, and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

#### Construction of pSOG19 and pSOG35

pSOG35 is a transformation vector which utilizes the *E. coli* gene dihydrofolate reductase (DHFR) as a selectable marker conferring resistance to methotrexate. PCR was used to amplify the 35S promoter (~800 bp), intron 6 from the maize *Adh1* gene (~350 bp) and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250 bp fragment encoding the *E. coli* dihydrofolate reductase type II gene was also amplified by PCR and these two PCR fragments were assembled with a *SacI*-*PstI* fragment from pBI221 (Clontech) which comprised the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generated pSOG19 which contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase terminator. Replacement of the GUS leader in

pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generated the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and have *HindIII*, *SphI*, *PstI* and *EcoRI* sites available for the cloning of foreign sequences such as chimeric gene sequences containing a plant protox promoter.

#### 5 EXAMPLE 6: Construction of Chimeric Genes/Plant Expression Cassettes

Coding sequences intended for expression in transgenic plants under the control of a plant protox promoter may be assembled in expression cassettes behind a suitable protox promoter and upstream of a suitable transcription terminator. The resulting chimeric genes can then be easily  
10 transferred to the plant transformation vectors described above in Example 19.

#### Prottox Promoter Selection

In accordance with the present invention, the chimeric gene will contain a plant protox promoter. The selection of the specific protox promoter used in the chimeric gene is primarily up  
15 to the individual researcher, although generally it will be preferable to use a protox promoter from a plant species closely related to, or most preferably identical, to the species intended to contain the resulting chimeric gene. For example, if the chimeric gene is intended to be contained in a maize plant it would be preferable to use a protox promoter from a monocotyledonous plant and most preferable to use a maize protox promoter.

#### Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those which are known to function  
25 in plants and include the CaMV 35S terminator, the *nml* terminator, the nopaline synthase terminator, the pea *rbcsE9* terminator, as well as terminators naturally associated with the plant protox gene (i.e. "prottox terminators"). These can be used in both monocotyledons and dicotyledons.

#### Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

- 5 Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adh1* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*,  
10 *Genes Develop.* 1: 1183-1200 (1987)). In the same experimental system, the intron from the maize *bronzel* gene had a similar effect in enhancing expression (Callis *et al.*, *supra*). Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

- A number of non-translated leader sequences derived from viruses are also known to  
15 enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (e.g. Callie *et al. Nucl. Acids Res.* 15: 8693-8711 (1987); Skuzeski *et al. Plant Molec. Biol.* 15: 65-79 (1990))

20

#### Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence  
25 found at the amino terminal end of various proteins and which is cleaved during chloroplast import yielding the mature protein (e.g. Cornai *et al. J. Biol. Chem.* 263: 15104-15109 (1988)). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck *et al. Nature* 313: 358-363 (1985)). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs

encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins which are known to be chloroplast localized.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (e.g. Unger *et al.* *Plant Molec. Biol.* 13: 411-418 (1989)). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting to cellular protein bodies has been described by Rogers *et al.* *Proc. Natl. Acad. Sci. USA* 82: 6512-6516 (1985).

In addition sequences have been characterized which cause the targeting of gene products to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from alveolar cells (Koehler & Ho, *Plant Cell* 2: 769-783 (1990)). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.* *Plant Molec. Biol.* 14: 357-368 (1990)).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the amino terminal ATG of the transgene. The signal sequence selected should include the known cleavage site and the fusion constructed should take into account any amino acids after the cleavage site which are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or alternatively replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques described by Bartlett *et al.* In: Edelman *et al.* (Eds.) *Methods in Chloroplast Molecular Biology*, Elsevier, pp 1081-1091 (1982); Wasmann *et al.* *Mol. Gen. Genet.* 205: 446-453 (1986)). These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes. The choice of targeting which may be required for expression

If the transgenes will depend on the cellular localization of the precursor required as the starting point for a given pathway. This will usually be cytosolic or chloroplasmic, although it may in some cases be mitochondrial or peroxisomal. The products of transgene expression will not normally require targeting to the ER, the apoplast or the vacuole.

The above described mechanisms for cellular targeting can be utilized in conjunction with plant protoplast promoters so as to effect a specific cell targeting goal under the transcriptional regulation of a promoter which has an expression pattern different to that of the promoter from which the targeting signal derives.

#### EXAMPLE 7: Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques which do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski *et al.*, *EMBO J* 3: 2717-2722 (1984), Potrykus *et al.*, *Mol. Gen. Genet.* 199: 169-177 (1985), Reich *et al.*, *Biotechnology* 4: 1001-1004 (1986), and Klein *et al.*, *Nature* 327: 70-73 (1987). In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

*Agrobacterium*-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. The many crop species which are routinely transformable by *Agrobacterium* include tobacco, tomato, sunflower, cotton, oilseed rape, potato, soybean, alfalfa and poplar (EP 0 317 511 (cotton), EP 0 249 432 (tomato, to Calgene), WO 87/07299 (Brassica, to Calgene), US 4,795,855 (poplar)).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.



**EXAMPLE 8: Transformation of Monocotyledons**

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, and particle bombardment into callus tissue. Transformations can be undertaken with a single

- 5 DNA species or multiple DNA species (i.e. co-transformation) and both these techniques are suitable for use with this invention. Co-transformation may have the advantage of avoiding complex vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded desirable. However, a disadvantage of the use of co-
- 10 transformation is the less than 100% frequency with which separate DNA species are integrated into the genome (Schocher *et al. Biotechnology* 4: 1093-1096 (1986)).

- Patent Applications EP 0 292 435 (to Ciba-Geigy), EP 0 392 225 (to Ciba-Geigy), WO 93/07278 (to Ciba-Geigy) and U.S. Patent No. 5,350,689 (to Ciba-Geigy) describe techniques for the preparation of callus and protoplasts from an elite inbred line of maize, transformation of
- 15 protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm *et al., Plant Cell* 2: 603-618 (1990)) and Fromm *et al., Biotechnology* 8: 833-839 (1990)) have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, application WO 93/07278 (to Ciba-Geigy) and Koziel *et al., Biotechnology* 11: 194-200 (1993)) describe techniques for the transformation
- 20 of elite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for bombardment.

- Transformation of rice can also be undertaken by direct gene transfer techniques utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been described for
- 25 Japonica-types and Indica-types (Zhang *et al., Plant Cell Rep* 7: 379-384 (1988); Shimamoto *et al., Nature* 338: 274-277 (1989); Datta *et al., Biotechnology* 8: 735-740 (1990)). Both types are also routinely transformable using particle bombardment (Christou *et al., Biotechnology* 9: 957-962 (1991)).

Patent Application EP 0 332 581 (to Ciba-Geigy) describes techniques for the generation, transformation and regeneration of Pooidae protoplasts. These techniques allow the transformation of *Dactylis* and wheat. Furthermore, wheat transformation was described by Vasil *et al.*, *Biotechnology* 10: 667-674 (1992) using particle bombardment into cells of type C long-term regenerable callus, and also by Vasil *et al.*, *Biotechnology* 11: 1553-1558 (1993) and Weeks *et al.*, *Plant Physiol.* 102: 1077-1084 (1993) using particle bombardment of immature embryos and immature embryo-derived callus. A preferred technique for wheat transformation, however, involves the transformation of wheat by particle bombardment of immature embryos and includes either a high sucrose or a high maltose step prior to gene delivery. Prior to bombardment, any number of embryos (0.75-1 mm in length) are plated onto MS medium with 3% sucrose (Murashige & Skoog, *Physiologia Plantarum* 15: 473-497 (1962)) and 3 mg/l 2,4-D for induction of somatic embryos which is allowed to proceed in the dark. On the chosen day of bombardment, embryos are removed from the induction medium and placed onto the osmoticum (i.e. induction medium with sucrose or maltose added at the desired concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 h and are then bombarded. Twenty embryos per target plate is typical, although not critical. An appropriate gene-carrying plasmid (such as pCIB3064 or pSOG35) is precipitated onto micrometer size gold particles using standard procedures. Each plate of embryos is shot with the DuPont Biolistics® helium device using a burst pressure of ~1000 psi using a standard 80 mesh screen. After bombardment, the embryos are placed back into the dark to recover for about 24 h (still on osmoticum). After 24 hrs, the embryos are removed from the osmoticum and placed back onto induction medium where they stay for about a month before regeneration. Approximately one month later the embryo explants with developing embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter GA), further containing the appropriate selection agent (10 mg/l basta in the case of pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one month, developed shoots are transferred to larger sterile containers known as "GA7s" which contained half-strength MS, 2% sucrose, and the same concentration of selection agent. Patent application 08/147,161 describes methods for wheat transformation and is hereby incorporated by reference.

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While the present invention has been described with reference to specific embodiments thereof, it will be appreciated that numerous variations, modifications, and embodiments are possible, and accordingly, all such variations, modifications and embodiments are to be regarded as being within the spirit and scope of the present invention.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: PROMOTERS FROM PLANT PROTOPORPHYRINOGEN  
OXIDASE GENES

(iii) NUMBER OF SEQUENCES: 14

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US TBA  
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(C) CLASSIFICATION:

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## (2) INFORMATION FOR SEQ ID NO:1:

(1) SEQUENCE CHARACTERISTICS  
(A) LENGTH: 1719 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 31..1644

15 (D) OTHER INFORMATION: /note= "Arabidopsis protax-1 cDNA:  
sequence from pBDC-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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30 AAT GTT TAT AAG CCT CTT AGA CTC CGT TGT TCA GTG GCC GGT GGA CCA      150
   Asn Val Tyr Lys Pro Leu Arg Leu Arg Cys Ser Val Ala Gly Gly Pro
   25          30          35          40

35 ACC GTC GGA TCT TCA AAA ATC GAA GGC GGA GGA ACC ACC ATC ACC      198
   Thr Val Gly Ser Ser Lys Ile Glu Gly Gly Gly Thr Thr Ile Thr
   45          50          55

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   75          80          85

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410 415 420

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 Lys

1719

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 517 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Leu Ser Leu Leu Arg Pro Thr Thr Gln Ser Leu Leu Pro Ser 1 5 10 15  
 Phe Ser Lys Pro Aen Leu Arg Leu Aen Val Tyr Lys Pro Leu Arg Leu 20 25 30  
 Arg Cys Ser Val Ala Gly Gly Pro Thr Val Gly Ser Ser Lys Ile Glu 35 40 45  
 Gly Gly Gly Gly Thr Thr Ile Thr Thr Asp Cys Val Ile Val Gly Gly 50 55 60  
 Gly Ile Ser Gly Leu Cys Ile Ala Gln Ala Leu Ala Thr Lys His Pro 65 70 75 80

Asp Ala Ala Pro Asn Leu Ile Val Thr Glu Ala Lys Asp Arg Val Gly  
 85 90 95  
 5 Gly Asn Ile Ile Thr Arg Glu Glu Asn Gly Phe Leu Trp Glu Glu Gly  
 100 105 110  
 Pro Asn Ser Phe Gln Pro Ser Asp Pro Met Leu Thr Met Val Val Asp  
 115 120 125  
 10 Ser Gly Leu Lys Asp Asp Leu Val Leu Gly Asp Pro Thr Ala Pro Arg  
 130 135 140  
 Phe Val Leu Trp Asn Gly Lys Leu Arg Pro Val Pro Ser Lys Leu Thr  
 145 150 155 160  
 15 Asp Leu Pro Phe Phe Asp Leu Met Ser Ile Gly Gly Lys Ile Arg Ala  
 165 170 175  
 Gly Phe Gly Ala Leu Gly Ile Arg Pro Ser Pro Pro Gly Arg Glu Glu  
 180 185 190  
 20 Ser Val Glu Glu Phe Val Arg Arg Asn Leu Gly Asp Glu Val Phe Glu  
 195 200 205  
 25 Arg Leu Ile Glu Pro Phe Cys Ser Gly Val Tyr Ala Gly Asp Pro Ser  
 210 215 220  
 Lys Leu Ser Met Lys Ala Ala Phe Cys Val Trp Lys Leu Glu Gln  
 225 230 235 240  
 30 Asn Gly Gly Ser Ile Ile Gly Gly Thr Phe Lys Ala Ile Gln Glu Arg  
 245 250 255  
 Lys Asn Ala Pro Lys Ala Glu Arg Asp Pro Arg Leu Pro Lys Pro Gln  
 260 265 270  
 35 Gly Gln Thr Val Gly Ser Phe Arg Lys Glu Leu Arg Met Leu Pro Glu  
 275 280 285  
 40 Ala Ile Ser Ala Arg Leu Gly Ser Lys Val Lys Leu Ser Trp Lys Leu  
 290 295 300  
 Ser Gly Ile Thr Lys Leu Glu Ser Gly Gly Tyr Asn Leu Thr Tyr Glu  
 305 310 315 320  
 45 Thr Pro Asp Gly Leu Val Ser Val Gln Ser Lys Ser Val Val Met Thr  
 325 330 335  
 Val Pro Ser His Val Ala Ser Gly Leu Leu Arg Pro Leu Ser Glu Ser  
 340 345 350  
 50 Ala Ala Asn Ala Leu Ser Lys Leu Tyr Tyr Pro Pro Val Ala Ala Val  
 355 360 365  
 55 Ser Ile Ser Tyr Pro Lys Glu Ala Ile Arg Thr Glu Cys Leu Ile Asp  
 370 375 380  
 Gly Glu Leu Lys Gly Phe Gly Gln Leu His Pro Arg Thr Gln Gly Val  
 385 390 395 400  
 60 Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn Arg Ala  
 405 410 415

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Pro Gly Arg Ile Leu Leu Leu Asn Tyr Ile Gly Gly Ser Thr Asn
420 425 430
Thr Gly Ile Leu Ser Lys Ser Glu Gly Glu Leu Val Glu Ala Val Asp
435 440 445
Arg Asp Leu Arg Lys Met Leu Ile Lys Pro Asn Ser Thr Asp Pro Leu
450 455 460
Lys Leu Gly Val Arg Val Trp Pro Gln Ala Ile Pro Gln Phe Leu Val
465 470 475
Gly His Phe Asp Ile Leu Asp Thr Ala Lys Ser Ser Leu Thr Ser Ser
485 490 495
Gly Tyr Glu Gly Leu Phe Leu Gly Gly Asn Tyr Val Ala Gly Val Ala
500 505 510
Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu Thr Ala Ile Glu Val Asn
515 520 525
Asn Phe Met Ser Arg Tyr Ala Tyr Lys
530 535

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(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1734 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 78..1596
(D) OTHER INFORMATION: /note= "Arabidopsis protease-2 cDNA:
sequence from pMDC-1"
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
50 TTTTACTT ATTCCTCA CAGCTTCA CAGTCAGAG ATTTGACTC TGAATGTTG 60
CAGATACCA ATG GCG TCT GGA GGA GTA GCA GAT CAT CAA ATT GAA GCG 108
Met Ala Ser Gly Ala Val Ala Asp His Gln Ile Glu Ala
1 5 10
55 GTT TCA GGA AAA AGA GTC GCA GTC GTA GGT GCA GGT GTA AGT GGA GTT 156
Val Ser Gly Lys Arg Val Ala Val Val Gly Ala Gly Val Ser Gly Leu
15 20 25
60 GCG GCG GCT TAC AAC TTG AAA TCG AGG GGT TTG AAT GTG ACT GTG TTT 204
Ala Ala Ala Tyr Lys Leu Lys Ser Arg Gly Leu Asn Val Thr Val Phe
30 35 40 45

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	1A	GCT	GAT	GGA	AGA	GTA	GCT	GCG	AAG	TTG	AGA	AGT	GTT	ATG	CAA	AAT	252
	4	Ala	Asp	Gly	Arg	Val	Gly	Gly	Lys	Leu	Arg	Ser	Val	Met	Gln	Asn	
					50					55					60		
5		GCT	TTG	ATT	TGG	GAT	GAA	GGA	OCA	AAC	ACC	ATG	ACT	GAG	GCT	GAG	300
		Gly	Leu	Ile	Trp	Asp	Glu	Gly	Ala	Asn	Thr	Met	Thr	Glu	Ala	Glu	
					65					70					75		
10		GAA	GTT	GCG	AGT	TTA	CTT	GAT	GAT	CTT	GCG	CTT	CGT	GAG	AAA	CAA	348
		Glu	Val	Gly	Ser	Leu	Leu	Asp	Asp	Leu	Gly	Leu	Arg	Glu	Lys	Gln	
					80					85					90		
15		TTT	CCA	ATT	TCA	CAG	AAA	AAG	GCG	TAT	ATT	GTG	CGG	AAT	GCT	GTA	396
		Phe	Pro	Ile	Ser	Gln	Lys	Arg	Tyr	Ile	Val	Arg	Asn	Gly	Val	Pro	
					95					100							
20		GTG	ATC	CTA	CCT	ACC	AAT	CCC	ATA	GAG	CTG	CTC	ACA	AGT	AGT	GTG	444
		Val	Met	Leu	Pro	Thr	Asn	Pro	Ile	Glu	Leu	Val	Thr	Ser	Ser	Val	
							115					120				125	
25		TCT	ACC	CAA	TCT	AAG	TTT	CAA	ATC	TTG	TTG	GAA	CCA	TTT	TTA	TGG	492
		Ser	Thr	Gln	Ser	Lys	Phe	Gln	Ile	Leu	Lau	Glu	Pro	Phe	Leu	Trp	
							130					135				140	
30		AAA	AAG	TCC	TCA	AAA	CTC	TCA	GAT	OCA	TCT	GCT	GAA	GAA	AGT	GTA	540
		Lys	Lys	Ser	Ser	Lys	Val	Ser	Asp	Ala	Ser	Ala	Glu	Glu	Ser	Val	
							145				150				155		
35		GAG	TTG	TTT	CAA	GCG	CAT	TTT	GGA	CAA	GAG	GTT	GTT	GAC	TAT	CTC	588
		Glu	Phe	Phe	Gln	Arg	His	Phe	Gly	Gln	Glu	Val	Val	Asp	Tyr	Leu	
										165					170		
40		GAC	CGT	TTT	GTT	GCT	GGA	ACA	AGT	GCT	GCG	GAC	CCT	GAT	TCC	CTT	636
		Asp	Pro	Phe	Val	Gly	Thr	Ser	Ala	Ala	Asp	Pro	Asp	Ser	Leu	Ser	
							175				180				185		
45		ATG	AAG	CAT	TCT	TTT	GCA	GAT	CTC	TGG	AAT	GTA	GAG	AAA	AGT	TTT	684
		Met	Lys	His	Ser	Phe	Pro	Asp	Leu	Trp	Asn	Val	Glu	Lys	Ser	Phe	
								195				200				205	
50		TCT	ATT	ATA	GTC	GCT	GCA	ATC	AGA	ACA	AAG	TTT	GCT	GCT	AAA	GCT	732
		Ser	Ile	Ile	Val	Gly	Ala	Ile	Arg	Thr	Lys	Phe	Ala	Ala	Lys	Gly	
								210				215				220	
55		AAA	AGT	AGA	GAC	ACA	AAG	AGT	TCT	CCT	GCG	ACA	AAA	AAG	GCT	TGG	780
		Lys	Ser	Arg	Asp	Thr	Lys	Ser	Ser	Pro	Gly	Thr	Lys	Lys	Gly	Ser	
								225			230				235		
60		GCG	TCA	TTT	TCT	TCT	AAG	GCG	GGA	ATC	CAG	ATT	CTT	CCT	GAT	ACC	828
		Gly	Ser	Phe	Ser	Phe	Lys	Gly	Gly	Met	Gln	Ile	Leu	Pro	Asp	Thr	
										245					250		
65		TGC	AAA	AGT	CTC	TCA	CAT	GAT	GAG	ATC	AAT	TTA	GAC	TCC	AAG	GTA	876
		Cys	Lys	Ser	Leu	Ser	His	Asp	Glu	Ile	Asn	Leu	Asp	Ser	Lys	Val	
								260				265					
70		TCT	TTG	TCT	TAC	AAT	TCT	GGA	TCA	AGA	CAG	GAG	AAC	TGG	TCA	TTA	924
		Ser	Leu	Ser	Tyr	Asn	Ser	Gly	Ser	Arg	Gln	Glu	Asn	Trp	Ser	Leu	
								275				280				285	
75		TCT	GTT	TGG	CAT	AAT	GAA	ACC	CAG	AGA	CAA	AAC	CCC	CAT	TAT	GAT	972
		Cys	Val	Ser	His	Asn	Glu	Thr	Gln	Arg	Gln	Asn	Pro	His	Tyr	Asp	
								290				295				300	

ATT ATG ACG GCT CCT CTG TCC AAT GTG AAG GAG ATG AAG GTT ATG 1020  
Val Ile Met Thr Thr Ala Pro Leu Cys Asn Val Lys Glu Met Lys Val Met  
305 310 315

5 AAA GGA GCA CAA CCC TTT CAG CTA AAC TTT CTC CCC GAG ATT AAT TAC 1068  
Lys Gly Gly Gln Pro Phe Gln Leu Asn Phe Leu Pro Glu Ile Asn Tyr  
320 325 330

10 ATG CCC CTC TCG GTT TTA ATC ACC ACA TTC ACA AAG GAG AAA GTA AAG 1116  
Met Pro Leu Ser Val Leu Ile Thr Thr Phe Thr Lys Glu Lys Val Lys  
335 340 345

AGA CCT CTT GAA GGC TTT GGG GTA CTC ATT CCA TCT AAG GAG CAA AAG 1164  
Arg Pro Leu Glu Gly Phe Gly Val Leu Ile Pro Ser Lys Glu Gln Lys  
350 355 360 365

15 CAT GGT TTC AAA ACT CTA GGT ACA CTT TTT TCA TCA ATG ATG TTT CCA 1212  
His Gly Phe Lys Thr Leu Gly Thr Leu Phe Ser Ser Met Met Phe Pro  
370 375 380

20 GAT GGT TCC CCT AGT GAC GTT CAT CTA TAT ACA ACT TTT ATT GGT GGG 1260  
Asp Arg Ser Ser Ser Asp Val His Leu Tyr Thr Thr Phe Ile Gly Gly  
385 390 395

25 AGT ACG AAC CAG GAA CTA GCT AAA GCT TCC ACT GAC CAA TTA AAA CAA 1308  
Ser Arg Asn Gln Glu Leu Ala Lys Ala Ser Thr Asp Glu Leu Lys Gln  
400 405 410

30 GTT GTG ACT TCT GAC CTT CAG CGA CTG TTG GGG GTT GAA GGT GAA CCC 1356  
Val Val Thr Ser Asp Leu Gln Leu Arg Leu Gly Val Gly Glu Pro  
415 420 425

35 GTG TCT GTC AAC CAT TAC TAT TCG AGG AAA GCA TTC CCG TTG TAT GAC 1404  
Val Lys Ser Val Asn His Tyr Tyr Trp Arg Lys Ala Phe Pro Leu Tyr Asp  
430 435 440 445

40 AGC AGC TAT GAC TCA GTC ATG GAA GCA ATT GAC AAG ATG GAG AAT GAT 1452  
Ser Ser Tyr Asp Ser Val Met Glu Ala Ile Asp Lys Met Glu Asn Asp  
450 455 460

CTA CCT GGG TTC TTC TAT GCA GGT AAT CAT CGA GGG GGG CTC TCT GGT 1500  
Leu Pro Gly Phe Phe Tyr Ala Gly Asn His Arg Gly Gly Leu Ser Val  
465 470 475

45 GGG AAA TCA ATA GCA TCA GGT TCC AAA GCA GCT GAC CTT GTG ATC TCA 1548  
Gly Lys Ser Ile Ala Ser Gly Cys Lys Ala Asp Leu Val Ile Ser  
480 485 490

50 TAC CTG GAG TCT TCC TCA AAT GAC AAG AAA CCA AAT GAC AGC TTA TAACATTGTC 1603  
Tyr Leu Glu Ser Cys Ser Asn Asp Lys Lys Pro Asn Asp Ser Leu  
495 500 505

AAGGTCGTC CTTTTTATC ACTTACTTG TAAACTGTA AAATGCAACA AGCGGCGGTC 1663

55 CGATTAGCCA ACAACTCAG: AAAACCCAGA TTCTCATAAG GCTCACTAAT TCCAGAAATTA 1723  
ACTATTATG TAAAA 1728

60

(2) INFORMATION FOR SEQ ID NO:4:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 508 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

5 (11) MOLECULE TYPE: protein

(m1) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ala Ser Gly Ala Val Ala Asp His Gln Ile Glu Ala Val Ser Gly
1      5      10      15
Lys Arg Val Ala Val Val Gly Ala Gly Val Ser Gly Leu Ala Ala Ala
20      25      30
Tyr Lys Leu Lys Ser Arg Gly Leu Asn Val Thr Val Phe Glu Ala Asp
35      40      45
Gly Arg Val Gly Gly Lys Leu Arg Ser Val Met Gln Asn Gly Leu Ile
50      55      60
Trp Asp Glu Gly Ala Asn Thr Met Thr Glu Ala Glu Pro Glu Val Gly
65      70      75      80
Ser Leu Leu Asp Asp Leu Gly Leu Arg Glu Lys Gln Gln Phe Pro Ile
85      90      95
Ser Gln Lys Lys Arg Tyr Ile Val Arg Asn Gly Val Pro Val Met Leu
100     105     110
Pro Thr Asn Pro Ile Glu Leu Val Thr Ser Ser Val Leu Ser Thr Gln
115     120     125
Ser Lys Phe Gln Ile Leu Leu Glu Pro Phe Leu Trp Lys Lys Lys Ser
130     135     140
Ser Lys Val Ser Asp Ala Ser Ala Glu Glu Ser Val Ser Glu Phe Phe
145     150     155     160
Gln Arg His Phe Gly Gln Glu Val Val Asp Tyr Leu Ile Asp Pro Phe
165     170     175
Val Gly Gly Thr Ser Ala Ala Asp Pro Asp Ser Leu Ser Met Lys His
180     185     190
Ser Phe Pro Asp Leu Trp Asn Val Glu Lys Ser Phe Gly Ser Ile Ile
195     200     205
Val Gly Ala Ile Arg Thr Lys Phe Ala Ala Lys Gly Gly Lys Ser Arg
210     215     220
Asp Thr Lys Ser Ser Pro Gly Thr Lys Lys Gly Ser Arg Gly Ser Phe
225     230     235     240
Ser Phe Lys Gly Gly Met Gln Ile Leu Pro Asp Thr Leu Cys Lys Ser
245     250     255
Leu Ser His Asp Glu Ile Asn Leu Asp Ser Lys Val Leu Ser Leu Ser
260     265     270
Tyr Asn Ser Gly Ser Arg Gln Glu Asn Trp Ser Leu Ser Cys Val Ser
275     280     285
His Asn Glu Thr Gln Arg Gln Asn Pro His Tyr Asp Ala Val Ile Met
32

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290                295                300
Thr Ala Pro Leu Cys Asn Val Lys Glu Met Lys Val Met Lys Gly Gly
305                310                315
5  Glu Pro Phe Gln Leu Asn Phe Leu Pro Glu Ile Asn Tyr Met Pro Leu
    325                330                335
Ser Val Leu Ile Thr Thr Phe Thr Lys Glu Lys Val Lys Arg Pro Leu
10 340                345                350
    Glu Gly Phe Gly Val Leu Ile Pro Ser Lys Glu Gln Lys His Gly Phe
    355                360                365
15  Lys Thr Leu Gly Thr Leu Phe Ser Ser Met Met Phe Pro Asp Arg Ser
    370                375                380
    Pro Ser Asp Val His Leu Tyr Thr Thr Phe Ile Gly Gly Ser Arg Asn
    385                390                395
20  Gln Glu Leu Ala Lys Ala Ser Thr Asp Glu Leu Lys Gln Val Thr
    405                410                415
    Ser Asp Leu Gln Arg Leu Leu Gly Val Glu Gly Pro Val Ser Val
25 420                425                430
    Asn His Tyr Tyr Trp Arg Lys Ala Phe Pro Leu Tyr Asp Ser Ser Tyr
    435                440                445
30  Asp Ser Val Met Glu Ala Ile Asp Lys Met Glu Asn Asp Leu Pro Gly
    450                455                460
    Phe Phe Tyr Ala Gly Asn His Arg Gly Gly Leu Ser Val Gly Lys Ser
    465                470                475
35  Ile Ala Ser Gly Cys Lys Ala Ala Asp Leu Val Ile Ser Tyr Leu Glu
    485                490                495
    Ser Cys Ser Asn Asp Lys Lys Pro Asn Asp Ser Leu
40 500                505

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## (2) INFORMATION FOR SEQ ID NO:5:

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45  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 1691 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear
50  (ii) MOLECULE TYPE: cDNA
    (iii) HYPOTHETICAL: NO
55  (iv) ANTI-SENSE: NO
    (ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 1..1443
    (D) OTHER INFORMATION: /note= "Maize protom-1
60  cDNA (not full-length): sequence from pMDC-4; first seven
    nucleotides removed vs. first provisional"
    33

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## (x1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

5	CGG GAC TGC CTC GTC GTG GGC GGA GGC ATC AGT GGC CTC TGC ACC CGG	48
	Ala Asp Cys Val Val Val Gly Gly Ile Ser Gly Leu Cys Thr Ala	
	1 5 19 15	
10	CAG GCG CTG GCC ACC CGG CAC GGC GTC GGG GAC GTG CTT GTC ACC GAG	96
	Gln Ala Leu Ala Thr Arg His Gly Val Gly Asp Val Leu Val Thr Glu	
	20 25 30	
15	GCC CGC CCC CGC CCC GGC GGC AAC ATT ACC ACC GTC GAG CGC CCC GAG	144
	Ala Arg Ala Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Glu	
	35 40 45	
20	GAA GGC PAC CTC TGC GAG GAG GGT CCC AAC AGC TTC CAG CCC TCC GAC	192
	Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp	
	50 55 60	
25	CCC GTT CTC ACC ATG GGC GTG GAC AOC GGA CTG AAG GAT GAC TTG GTT	240
	Phe Gly Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val	
	65 70 75 80	
30	TTT GCG GAC CCA AAC GGC CGC CGT TTC GTG CTG TCG GAG GGC AAG CTG	288
	Pro Val Leu Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu	
	85 90 95	
35	AGG CCC GTG CCA TCC AAG CCC GGC GAC CTC CGC TTC TTC GAT CTC ATG	336
	Arg Pro Val Pro Ser Lys Pro Ala Asp Leu Pro Phe Phe Asp Leu Met	
	100 105 110	
40	AGC ATC CCA GCG AAG CTC ACG GCT GGT CTA GGC CGC GGT GGC ATC CGC	384
	Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg	
	115 120 125	
45	CCG COT CCT CCA GGC CGC GAA GAG TCA GTG GAG GAG TTC GTG CGC CGC	432
	Pro Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg	
	130 135 140	
50	AAC CTC GGT GCT GAG GTC TTT GAG CGC CTC ATT GAG CTT TTC TGC TCA	480
	Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser	
	145 150 155 160	
55	GCT GTC TAT GCT GGT GAT CCT TCT AAG CTC AOC ATG AAG GCT GCA TTT	528
	Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe	
	165 170 175	
60	GCG AAG GTT TCG CGC TTG GAA GAA ACT GCA GGT AGT ATT ATT GGT GGA	576
	Gly Lys Val Trp Arg Leu Glu Glu Thr Gly Gly Ser Ile Ile Gly Gly	
	180 185 190	
65	ACC ATC AAG ACA ATT CAG GAG AGC AGC AAG AAT CCA / / A CCG AGG	624
	Thr Ile Lys Thr Ile Gln Glu Arg Ser Lys Asn Pro Lys Pro Pro Arg	
	195 200 205	
70	GAT GGC CGC CTT CGC AAG CCA AAA GGC CAG ACA GTT GCA TCT TTC AGG	672
	Asp Ala Arg Leu Pro Lys Pro Lys Gly Gln Thr Thr Val Ala Ser Phe Arg	
	210 215 220	
75	AAG GTT CTT GGC ATG CTT CCA AAT GCT ATT ACA TCC AGC TTC GGT AGT	720
	Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Thr Ser Ser Leu Gly Ser	
	225 230 235 240	

	AAU AC AAA CTA TCA TCG AAA CTC AGC AGC ATT ACA AAA TCA GAT GAC	768
	Lys Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ser Asp Asp	
	245 250	
5	AAG GCA TAT GTT TTG GAG TAT GAA AGC CCA GAA GGC GTT GTT TCG GTC	816
	Lys Gly Tyr Val Leu Glu Thr Glu Thr Pro Glu Gly Val Val Ser Val	
	260 265 270	
10	CAG GCT AAA AGT GTT ATC ATG AGT ATT CCA TCA TAT GTT GCT AGC AAC	864
	Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asn	
	275 280 285	
	ATT TTG CGT CCA CTT TCA AGC GAT GCT GCA GAT GCT CTA TCA AGA TTC	912
15	Ile Leu Arg Pro Leu Ser Ser Asp Ala Ala Asp Ala Leu Ser Arg Phe	
	290 295 300	
	TAT TAT CCA CCG GTT GCT GCT GTA ACT GTT TCG TAT CCA AAG GAA GCA	960
20	Tyr Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala	
	305 310 315 320	
	ATT AGA AAA GAA TGC TTA ATT GAT GGC GAA CTC CAG GGC TTT GGC CAG	1008
	Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln	
	325 330 335	
25	TTG GAT CCA CGT AGT CAA GGA GTT GAG ACA TTA GCA ACA ATA TAC AGT	1056
	Leu His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Tyr Ser	
	340 345 350	
30	TCC TCA CTC TTT CCA AAT CGT GCT CCT GAC GGT AGC GTC TTA CTT CTA	1104
	Ser Ser Leu Phe Pro Asn Arg Ala Pro Asp Gly Arg Val Leu Leu Leu	
	355 360 365	
35	AAC TAC ATA GGA GGT GCT ACA AAC ACA GCA ATT GTT TCC AAG ACT GAA	1152
	Asn Tyr Ile Gly Gly Ala Thr Asn Thr Gly Ile Val Ser Lys Thr Glu	
	370 375 380	
	AGT GAG CTG GTC GAA GCA GTT GAC CGT GAC CTC CGA AAA ATG CTT ATA	1200
40	Ser Glu Leu Val Glu Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile	
	385 390 395 400	
	AAT TCT ACA GCA CTG GAC CCT TTA CTC CTT GGT GTT CCA GTT TGG CCA	1248
	Asn Ser Thr Ala Val Asp Pro Leu Val Leu Gly Val Arg Val Trp Pro	
	405 410 415	
45	GAA GCC ATA CTT CAG TTC CTG GTA GGA CAT CTT CAT CTT CTG GAA GCC	1296
	Gln Ala Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Glu Ala	
	420 425 430	
50	GCA AAA GCT GCC CTG GAC CGA GGT GGC TAC GAT GGC CTG TTC CTA GGA	1344
	Ala Lys Ala Ala Leu Asp Arg Gly Tyr Asp Gly Leu Phe Leu Gly	
	435 440 445	
	GGG AAG TAT GTT GCA GGA GTT GGC CTG GGC AGA TGC GTT GAC GGC GGC	1392
55	Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala	
	450 455 460	
	TAT GAA AGT GCC TCG CAA ATA TCT GAC TTC TTG ACC AAG TAT GCC TAC	1440
60	Tyr Glu Ser Ala Ser Gln Ile Ser Asp Phe Leu Thr Lys Tyr Ala Tyr	
	465 470 475 480	
	AAG TGAATGAAGA AGTGAAGCCG TACTTTGTTAA TCGTTATGT TGCATAGATG	1493
	Lys	

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ATGCTCTCC CGCGAAGAAA AACCTTGAAT AGTATTTTIT ATTCTTATTT TGTAAATTCG  
 ATTCTCTGTC TTTTCTCTAT CAGTAATTAG TTATATTTTA GTCTCTAGAG AGATTCTCTT  
 GTTCACTGCC CTTCAGAAAGA AATTTTATTT TTCTATCTTT TATGAGAGCT GTGCTACTTA  
 AAAAAAAAAA AAAAAAAAAA

1553  
1613  
1673  
1691

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 481 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Asp Cys Val Val Val Gly Gly Gly Ile Ser Gly Leu Cys Thr Ala  
 1 5 10 15  
 Gln Ala Leu Ala Thr Arg His Gly Val Gly Asp Val Leu Val Thr Glu  
 20 25 30  
 Ala Arg Ala Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Glu  
 35 40 45  
 Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp  
 50 55 60  
 Pro Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val  
 65 70 75 80  
 Phe Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu  
 85 90 95  
 Arg Pro Val Pro Ser Lys Pro Ala Asp Leu Pro Phe Phe Asp Leu Met  
 100 105 110  
 Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg  
 115 120 125  
 Pro Pro Pro Pro Gly Arg Gly Glu Ser Val Glu Glu Phe Val Arg Arg  
 130 135 140  
 Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser  
 145 150 155 160  
 Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe  
 165 170 175  
 Gly Lys Val Trp Arg Leu Glu Thr Gly Gly Ser Ile Ile Gly Gly  
 180 185 190  
 Thr Ile Lys Thr Ile Gln Glu Arg Ser Lys Asn Pro Lys Pro Pro Arg  
 195 200 205  
 Asp Ala Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Ala Ser Thr Arg  
 210 215 220

Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Thr Ser Ser Leu Gly Ser  
 230 235 240  
 5 Lys Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ser Asp Asp  
 245 250 255  
 Lys Gly Tyr Val Leu Glu Tyr Glu Thr Pro Glu Gly Val Val Ser Val  
 260 265 270  
 10 Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asn  
 275 280 285  
 Ile Leu Arg Pro Leu Ser Ser Asp Ala Ala Asp Ala Leu Ser Arg Phe  
 290 295 300  
 Tyr Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala  
 305 310 315 320  
 20 Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln  
 325 330 335  
 Leu His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser  
 340 345 350  
 25 Ser Ser Leu Phe Pro Asn Arg Ala Pro Asp Gly Arg Val Leu Leu Leu  
 355 360 365  
 Asn Tyr Ile Gly Gly Ala Thr Asn Thr Gly Ile Val Ser Lys Thr Glu  
 370 375 380  
 Ser Glu Leu Val Glu Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile  
 385 390 395 400  
 35 Asn Ser Thr Ala Val Asp Pro Leu Val Leu Gly Val Arg Val Trp Pro  
 405 410 415  
 Gln Ala Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Glu Ala  
 420 425 430  
 40 Ala Lys Ala Ala Leu Asp Arg Gly Gly Tyr Asp Gly Leu Phe Leu Gly  
 435 440 445  
 Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala  
 450 455 460  
 45 Tyr Glu Ser Ala Ser Gln Ile Ser Asp Phe Leu Thr Lys Tyr Ala Tyr  
 465 470 475 480  
 50 Lys

(2) INFORMATION FOR SEQ ID NO:7:

- (1) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2061 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

((1)) MOLECULE TYPE: cDNA



(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 64..1698

10 (D) OTHER INFORMATION: /note= "Maize protox-2 cDNA;  
sequence from pMDC-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

15 CTCTCTTACC TCCACCTCCA CGACACCAAG CAATATCCCA TCCAGTTCCA AACCTTAAC 60  
 GAA ATG CTC GCT TGG ACT GGC TCA GGC TCA TCC OCT TGG TCC CAT GCT 108  
 Met Leu Ala Leu Thr Ala Ser Ala Ser Ser Ala Ser His Pro 15  
 20 TAT GCG CAC GGC TCC GCG CAC ACT CGT GCG CCC GCG CTA GGT GCG GTC 156  
 Tyr Arg His Ala Ser Ala His Thr Arg Arg Pro Arg Leu Arg Ala Val 30  
 25 CTC GCG ATG GCG GGC TCC GAC GAC CCC CGT GCA GCG CCC GCG AGA TCG 204  
 Leu Ala Met Ala Gly Ser Asp Asp Pro Arg Ala Ala Pro Ala Arg Ser 35  
 30 GTC GCG GTC GTC GGC GCG GTC AGC GCG GCG GCG TAC AGG 252  
 Val Ala Val Val Gly Ala Gly Val Ser Gly Leu Ala Ala Tyr Arg 50  
 35 CTC AGA CAG AGC GCG GTG AAC GTA AGC GTG TTC GAA GCG GCG GAC AGG 300  
 Leu Arg Gln Ser Gly Val Asn Val Thr Val Phe Glu Ala Ala Asp Arg 65  
 40 GCG GGA GGA AAG ATA CCG ACT AAT TCC GAG GCG GCG TTT GTC TGG GAT 348  
 Ala Gly Gly Lys Ile Arg Thr Asn Ser Glu Gly Gly Phe Val Trp Asp 80  
 45 GAA GGA GCT AAC ACC ATG ACA GAA GGT GAA TGG GAG GCG AGT AGA CTG 396  
 Glu Gly Ala Asn Thr Met Thr Glu Gly Glu Trp Glu Ala Ser Arg Leu 100  
 50 ATT GAT GAT CTT GGT CTA CAA GAC AAA CAG CAG TAT CCT AAC TCC CAA 444  
 Ile Asp Asp Thr Gly Leu Gln Asp Lys Gln Gln Tyr Pro Asn Ser Gln 115  
 55 CAC AAG COT TAC ATT GTC AJA GAT GGA GCA GCA CTG ATT CCT TCG 492  
 His Lys Arg Tyr Ile Val Lys Asp Gly Ala Pro Ala Leu Ile Pro Ser 130  
 60 GAT CCC ATT TCG CTA ATG AAA AGC AGT GTT CTT TCG ACA AAA TCA AAG 540  
 Asp Pro Ile Ser Leu Met Lys Ser Ser Val Leu Ser Thr Lys Ser Lys 145  
 65 ATT GCG TTA TTT TTT GAA CCA TTT CTC TAC AAG AAA GCT AAC ACA AGA 588  
 Ile Ala Leu Phe Phe Glu Trp Phe Leu Tyr Lys Lys Ala Asn Thr Arg 160  
 70 AAC TCT GGA AAA CTG TCT GAG GAG CAC TTG AGT GAG AGT GTT GCG AGC 636  
 Asn Ser Gly Lys Val Ser Glu Glu His Leu Ser Glu Ser Val Gly Ser 180

38

T TGT GAA GGC CAC TTT GGA AGA GAA GTT GTT GAC TAT TTT GTT GAT 684  
 2. Cys Glu Arg His Phe Gly Arg Glu Val Asp Tyr Phe Val Asp 205  
 5 CCA TTT GTA GCT GGA ACA AGT GCA GGA GAT CCA GAG TCA CTA TCT ATT 732  
 Pro Phe Val Ala Gly Thr Ser Ala Gly Asp Pro Glu Ser Leu Ser Ile 210  
 10 CGT CAT GCA TTC CCA GCA TTG TGG AAT TTG GAA AGA AAG TAT GGT CCA 780  
 Arg His Ala Phe Pro Ala Leu Trp Asn Leu Glu Arg Lys Tyr Gly Ser 225  
 15 GTT ATT GTT GGT GCC ATC TTG TCT AAG CTA GCA GCT AAA GGT GAT CCA 828  
 Val Ile Val Val Gly Ala Ile Leu Ser Lys Leu Ala Lys Gly Asp Pro 240  
 20 GTA AAG ACA AGA CAT GAT TCA TCA GGC AAA AGA AGC AAT AGA CCA GTG 876  
 Val Lys Thr Arg His Asp Ser Ser Gly Lys Arg Arg Asn Arg Arg Val 260  
 25 TCG TTT TCA TTT CAT GGT GGA ATG CAG TCA CTA ATA AAT GCA CTT CAC 924  
 Ser Phe Ser Phe His Gly Gly Met Gln Ser Leu Ile Asn Ala Leu His 275  
 30 AAT GAA GTT GGA GAT GAT AAT GTG AAG CTT GGT ACA GAA GTG TTG TCA 972  
 Asn Glu Val Val Gly Asp Asp Asn Val Lys Leu Gly Thr Glu Val Ser 290  
 35 TTG GCA TGT ACA TTT GAT GGA GTT CCT GCA CTA GGC AGC TGC TCA ATT 1020  
 Leu Ala Cys Thr Phe Asp Gly Val Pro Ala Leu Gly Arg Trp Ser Ile 305  
 40 TCT GTT GAT TCG AAG GAC GGT GAC AAG GAC CTT GCT AGT AAC CAA 1068  
 Ser Val Asp Ser Lys Asp Ser Gly Asp Lys Asp Leu Ala Ser Asn Gln 320  
 45 ACC TTT GAT GCT GTT ATA ATG ACA GCT CCA TTG TCA AAT GTC CGG AGG 1116  
 Thr Phe Asp Ala Val Ile Met Thr Ala Pro Leu Ser Asn Val Arg Arg 340  
 50 ATG AAG TTC ACC AAA GGT GGA GCT CCG GTT GTT CTT GAC TTT CTT CTT 1164  
 Met Lys Phe Thr Lys Gly Gly Ala Pro Val Val Leu Asp Phe Leu Pro 355  
 55 AAG ATG CAT TAT CTA CCA CTA TCT CTC ATG GTG ACT GCT TTT AAG AAG 1212  
 Lys Met Asp Tyr Leu Pro Leu Ser Ser Leu Met Val Thr Ala Phe Lys Lys 370  
 60 GAT GAT GTC AAG AAA CCT CTG GAA GGA TTT GGG GTC TTA ATA CCT TAC 1260  
 Asp Asp Val Lys Lys Pro Leu Glu Gly Phe Gly Val Leu Ile Pro Tyr 385  
 65 AAG GAA CAG CAA AAA CAT GGT CTG AAA ACC CTT GGG ACT CTC TTT TCC 1308  
 Lys Glu Gln Gln Lys His Gly Leu Lys Thr Leu Gly Thr Leu Phe Ser 400  
 70 TCA ATG ATG TTC CCA GAT CGA GCT GCT GAT GAC CAA TAT TTA TAT ACA 1356  
 Ser Met Met Phe Pro Asp Arg Ala Pro Asp Asp Gln Tyr Leu Tyr Thr 420  
 75 ACA TTT GTT GGG GGT AGC CAC AAT AGA GAT CTT GGT GCA GCT CCA AGC 1404  
 Thr Phe Val Gly Gly Ser His Asn Arg Asp Leu Ala Gly Ala Pro Thr 435

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          435          440          445
TCT ATT CTG AAA CAA CTT GTG ACC TCT GAC CTT AAA AAA CTC TTG GGC 1454
Ser Ile Leu Lys Gln Leu Val Thr Ser Asp Leu Lys Lys Leu Leu Gly
          450          455          460
5  GTA GAG GGG CAA CCA ACT TTT GTC AAG CAT GTA TAC TGG GGA AAT GCT 1500
Val Glu Gly Gln Pro Thr Phe Val Lys His Val Tyr Trp Gly Asn Ala
          465          470          475
10 TTT CCT TTG TAT GGC CAT GAT TAT AGT TCT GTA TTG GAA GGT ATA GAA 1548
Phe Pro Leu Tyr Gly His Asp Tyr Ser Ser Val Leu Glu Ala Ile Glu
          480          485          490          495
15 AAG ATG GAG AAA AAC CTT CCA GGG TTC TTC TAC GCA GGA AAT AGC AAG 1596
Phe Met Glu Lys Asn Leu Pro Gly Phe Tyr Ala Gly Asn Ser Lys
          500          505          510          515
20 GAT GGC CTT GCT GTT GGA AGT GTT ATA OCT TCA GGA AGC AAG GCT GCT 1644
Asp Gly Leu Ala Val Gly Ser Val Ile Ala Ser Gly Ser Lys Ala Ala
          520          525          530          535
25 GAC CTT GCA ATC TCA TAT CTT GAA TCT CAC ACC AAG CAT AAT AAT TCA 1692
Asp Leu Ala Ile Ser Tyr Leu Glu Ser His Thr Lys His Asn Asn Ser
          540          545          550          555
CAT TGAAAGTGTG TGACCTATCC TCTACAGATT GTGACAAAT TTCTCAGATT 1745
His
          560          565          570          575
30 CATCTACAGT AGAAACGAT GCGTTCAGT TTCAGAACAT CTTCACTTCT TCAGATATTA 1805
ACCGTTCGTT GAACATCCAC CAGAAAGGTA GTCACATGTG TAGGTGGGAA AATGAGGTTA 1865
AAAACTATTTA TGGCGGGCCA AATGTTCTCT TTGTTTTC TCACAGTGG CCTACGACAC 1925
TTGATGTTGG AAATGACATT AAATTTGTTG AATTGTTTGA GAACACATCG GTGACGTGTA 1985
ATATTGCGCT ATTGTGATT TAGCAATAGT CTTGGCCAGA TTATGCTTTA CCGCTTTAAA 2045
AAAAAAAAAA AAAAAA 2061

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## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1811 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..1589
- (D) OTHER INFORMATION: /product= "wheat protex-1 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

1 CCA ACA ATG GCC ACC GCC ACC GTC GCG GCG GCG TCG CCG CTC CCG 47  
 Ala Thr Met Ala Thr Ala Thr Val Ala Ala Ser Pro Leu Arg  
 1 5 10 15  
 5 GCG AGG GTC ACC GCG CCG CCA CAC CCG GTC CCG CCG COT TGC GCT ACC 95  
 Gly Arg Val Thr Gly Arg Pro His Arg Val 25  
 20 25 30 35  
 10 GCG AGC AGC TCG ACC GAG ACT CCG GCG GCG CCG GCG GTG CCG CTG TCC 143  
 Ala Ser Ser Ala Thr Glu Thr Pro Ala Ala Pro Gly Val Arg Leu Ser  
 35 40 45 50  
 15 GCG GAA TCG GTC ATT GTG GCG GCG GCG GCG GCG GTC TCG ACC GCG 191  
 Ala Glu Cys Val Ile Val Gly Ala Gly Ile Ser Gly Leu Cys Thr Ala  
 50 55 60 65  
 20 CAG GCG CTG GCC ACC CGA TAC GCG GTC AGC GAC CTG CTC GTC ACC GAG 239  
 Gln Ala Leu Ala Thr Arg Tyr Gly Val Ser Asp Leu Val Thr Glu  
 65 70 75 80  
 25 GCG CCG GAC CCG CCG GCG GCG AAC ATC ACC ACC GTC GAG CCG CCG GAC 287  
 Ala Arg Asp Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Asp  
 80 85 90 95  
 30 GAG GCG TAC CTG TCG GAG GAG GCG AAC AGC GTC CAG CCG TCC GAC 335  
 Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp  
 100 105 110 115  
 35 GCG GTC CTC ACC ATG GCC GTG GAC AGC GCG CTC AAG GAT GAC TTG GTC 383  
 Pro Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Leu Val  
 115 120 125 130  
 40 TTC GCG GAC CCG AAC GCG CCG CCG TTC GTG CTC TCG GAG GCG AAG CTG 431  
 Phe Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu  
 135 140 145 150  
 45 AGC CCG GTC CCG TCG AAG CCA GCG GAC CTG CCG TTC TTC AGC CTC ATG 479  
 Arg Pro Val Pro Ser Lys Pro Gly Asp Leu Pro Phe Phe Ser Leu Met  
 155 160 165 170  
 50 AGT ATC CCG GCG AAG CTC AGC GCG GCG CTT GCG GCG CTC GCG ATT CCG 527  
 Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg  
 175 180 185 190  
 55 CCA CCG CCG CCA GCG CCG GAG GAG TCG GTG GAG GAG TTC GTG CCG CCG 575  
 Pro Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg  
 195 200 205 210  
 60 AAC CTC GGT GCG GAG CTC TTC GAG CCG CTC ATC GAG CCG TTC TGC TCA 623  
 Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser  
 215 220 225 230  
 65 GGT GTA TAT GGT GAT CCG TCG AAG CTT AGT ATG AAG GGT GCA TTC 671  
 Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Phe  
 235 240 245 250  
 70 GCG AAG GTC TCG AGC TTG GAG GAG ATT GGA GGT AGT ATT ATT GGT GGA 719  
 Gly Lys Val Trp Arg Leu Glu Glu Ile Gly Gly Ser Ile Ile Gly Gly  
 255 260 265 270  
 75 ACC ATC AAG GCG ATT CAG GAT AAA GCG AAG AAC CCG AAA CCG CCA AGG 767  
 Thr Ile Lys Ala Ile Glu Asp Lys Gly Lys Asn Pro Lys Pro Pro Arg  
 275 280 285 290  
 41

	240		245		250		255	
	AT CCC CGA CTT CCG GCA CCA AAG GGA CAG AGG GTG GCA TCT TTC AGG		Pro Ala Pro Lys Gly		Thr Val Ala Ser Phe Arg		270	815
5	Asp Pro Arg Leu		260		265			
	AAG GGT CTA GCC ATG CTC CCG AAT GCC ATC GCA TCT AGG CTG AGT		Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Ala Ser Arg Leu Gly Ser		280		285	863
10	Lys Val Lys Leu Ser Trp Lys Leu Thr		295		300			
	AAA GTC AAG CTG TCA TGG AAG CTT ACC AGC ATT ACA AAG GCG GAC AAC		Lys Val Lys Leu Ser Trp Lys Leu Thr		315		320	911
15	CAG GGT AAA AGT GGT ATC ATG ACC ATC CCG TCA TAT GTT GCT AGT GAT		Gln Gly Tyr Val Leu Gly Glu Thr		330		335	959
20	CAG GGT AAA AGT GGT ATC ATG ACC ATC CCG TCA TAT GTT GCT AGT GAT		Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asp		345		350	1007
25	ATC TTG CCG CCA CTT TCA ATT GAT GCA GCA GAT GCA CTC TCA AAA TTC		Ile Leu Arg Pro Leu Ser Ile Asp Ala Ala Asp Ala Leu Ser Lys Phe		360		365	1055
	TAT TAT CCG CCA CTT GCT GCT GCT GTA ACT GTT TCA TAT CCA AAA GAA GCT		Tyr Tyr Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala		375		380	1103
30	ATT AGA AAA GAA TGC TTA ATT GAT GGG GAG CTC CAG GGT TTC GGC CAG		Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln		395		400	1151
35	TTG CAT CCA COT AGC CAA GGA GTC GAG ACT TTA GCG ACA ATA TAT AGC		Leu His Pro Arg Ser Gln Gly Val Glu Thr		410		415	1199
40	TCT TCT CTC TTT CTT AAT COT GCT CCT GCT GCA AGA CTC TTA CTT CTG		Ser Ser Leu Phe Pro Asn Arg Ala Pro Ala Gly Arg Val Leu Leu Leu		425		430	1247
45	AAC TAT ATC GGC GTT TCT ACA AAT ACA GGG ATC GTC TCC AAG ACT GAG		Asn Tyr Ile Gly Gly Ser Thr Asn Thr Thr Gly Ile Val Ser Lys Thr Glu		440		445	1295
	AGT GAC TTA GTA GGA GCC GTT GAC COT GAC CTC AGA AAA ATG TTG ATA		Ser Asp Leu Val Gly Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile		455		460	1343
50	AAC COT AGA GCA GCA GAC COT TTA GCA TTA GCG GTT GCA GTC TGG CCA		Asn Pro Arg Ala Ala Asp Pro Leu Ala Leu Gly Val Arg Val Trp Pro		465		470	1391
55	CAA GCA ATA CCA CAG TTT TTG ATT GCG CAC CTT GAT CCG CTT GCT GCT		Gln Ala Ile Pro Gln Phe Leu Ile Gly His Leu Asp Arg Leu Ala Ala		475		480	1439
60	GCA AAA TCT GCA CTG GGC CAA GCG GCG TAC GAC GCG TTG TTC CTA GTA		Ala Lys Ser Ala Leu Gly Gln Gly Tyr Asp Gly Leu Phe Leu Gly		485		490	1487
	GGA AAC TAC GTC GCA GGA GTT GCG TTG GCG CCA TGC ATC GAG GGT GCG				495			1535

Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Ile Glu Gly Ala  
 500 505 510  
 5 AAC GAG AGT GCC TCA CAA GTA TCT GAC TTC TTG ACC AAG TAT GCC TAC 1583  
 Tyr Glu Ser Ala Ser Glu Val Ser Asp Phe Leu Thr Lys Tyr Ala Tyr  
 515 520 525  
 10 AAG TGA TGGAGTAGT GCATCTCTTC ATTTTGTTC ATATACGAGG TGAGGCTAGG 1639  
 Lys \*  
 ATCGGTAAAC CATCATGAGA TTCTGTAGTG TTCTTTAAT TGAAAAACA AATTATTG 1699  
 ATGCAATATG TGCTCTTTTC TGTAGTTCCA GCATGTACAT CGGTATGGGA TAAAGTAGAA 1759  
 15 TAGCTATTC TGCAAAAGCA GTGATTTTTT TTGAAAAAAA AAAAAAAAAA AA 1811

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 529 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:10:

30 Ala Thr Met Ala Thr Ala Thr Val Ala Ala Ser Pro Leu Arg Gly  
 1 5 10 15  
 Arg Val Thr Gly Arg Pro His Arg Val Arg Pro Arg Cys Ala Thr Ala  
 20 25 30  
 35 Ser Ser Ala Thr Glu Thr Pro Ala Ala Pro Gly Val Arg Leu Ser Ala  
 35 40 45  
 Glu Cys Val Ile Val Gly Ala Gly Ile Ser Gly Leu Cys Thr Ala Glu  
 50 55 60  
 40 Ala Leu Ala Thr Arg Tyr Gly Val Ser Asp Leu Leu Val Thr Glu Ala  
 65 70 75 80  
 45 Arg Asp Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Asp Glu  
 85 90 95  
 Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Glu Pro Ser Asp Pro  
 100 105 110  
 50 Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val Phe  
 115 120 125  
 Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu Arg  
 130 135 140  
 55 Pro Val Pro Ser Lys Pro Gly Asp Leu Pro Phe Phe Ser Leu Met Ser  
 145 150 155 160  
 60 Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg Pro  
 165 170 175  
 Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn  
 43

180 185 190

Leu Gly Ala Glu Val Phe Glu Arg 200 Leu Ile Glu Pro Phe Cys Ser Gly 205

5 Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly 210 220

Lys Val Trp Arg Leu Glu Glu Ile Gly Ser Ile Ile Gly Gly Thr 225 230 235 240

10 Ile Lys Ala Ile Gln Asp Lys Gly Lys Asn Pro Lys Pro Pro Arg Asp 245 250 255

15 Pro Arg Leu Pro Ala Pro Lys Gly Gln Thr Val Ala Ser Phe Arg Lys 260 265 270

Gly Leu Ala Met Leu Pro Asn Ala Ile Ala Ser Arg Leu Gly Ser Lys 275 280 285

20 Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ala Asp Asn Gln 290 295 300

Gly Tyr Val Leu Gly Tyr Glu Thr Pro Glu Gly Leu Val Ser Val Gln 305 310 315 320

25 Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asp Ile 325 330 335

30 Leu Arg Pro Leu Ser Ile Asp Ala Ala Asp Ala Leu Ser Lys Phe Tyr 340 345 350

Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala Ile 355 360 365

35 Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln Leu 370 375 380

His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser 385 390 395 400

Ser Leu Phe Pro Asn Arg Ala Pro Ala Gly Arg Val Leu Leu Asn 405 410 415

45 Tyr Ile Gly Gly Ser Thr Asn Thr Gly Ile Val Ser Lys Thr Glu Ser 420 425 430

Asp Leu Val Gly Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile Asn 435 440 445

50 Pro Arg Ala Ala Asp Pro Leu Ala Leu Gly Val Arg Val Trp Pro Gln 450 455 460

Ala Ile Pro Gln Phe Leu Ile Gly His Leu Asp Arg Leu Ala Ala Ala 465 470 475 480

Lys Ser Ala Leu Gly Gln Gly Tyr Asp Gly Leu Phe Leu Gly Gly 485 490 495

60 Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Ile Glu Gly Ala Tyr 500 505 510

Glu Ser Ala Ser Gln Val Ser Asp Phe Leu Thr Lys Tyr Ala Tyr Lys

515

520

525

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1847 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 55..1683
- (D) OTHER INFORMATION: /product= "soybean protox-1 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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25  CTTTGACCA GTTTGGAAGA TAAGCAACGA ATAGTGCCAT TACTGTAAACC AACCT 57
    Met 355

30  GTT TCC GTC TTC AAC GAG ATC CTA TTC CCG CCG AAC CAA ACC CTT CTT 105
    Val Ser Val Phe Asn Glu Ile Leu Phe Pro Pro Asn Gln Thr Leu Leu
    360

35  CCG CCC TCC CTC CAT TCC CCA ACC TCT TTC ACC TCT CCC ACT CGA 153
    Arg Pro Ser Leu His Ser Pro Thr Ser Phe Phe Thr Ser Pro Thr Arg
    375

40  AAA TTC CCT CCG TCT CCC CCT AAC CCT ATT CTA CCG TGC TCC ATT GCG 201
    Lys Phe Pro Arg Ser Arg Pro Asn Pro Ile Leu Arg Cys Ser Ile Ala
    390

45  GAG GAA TCC ACC GCG TCT CCG CCC AAA ACC AGA GAC TCC CCC CCC GTG 249
    Glu Glu Ser Thr Ala Ser Pro Pro Lys Thr Arg Asp Ser Ala Pro Val
    405

50  GAC TGC GTC GTC GTC GCG GGA GGC GTC AGC GGC CTC TGC ATC GCC CAG 297
    Asp Cys Val Val Val Gly Gly Gly Val Ser Gly Leu Cys Ile Ala Gln
    420

55  GGC CTC GCC ACC AAA CAC GCC AAT GCC AAC GTC GTC GTC ACG GAG GCC 345
    Ala Leu Ala Thr Lys His Ala Asn Ala Asn Val Val Val Thr Glu Ala
    440

60  CGA GAC CCC CTC GCG GGC AAC ATC ACC ACG ATG GAG ACG GAC GGA TAC 393
    Arg Asp Arg Val Gly Gly Asn Ile Thr Thr Met Glu Arg Asp Gly Tyr
    455

65  CTC TGG GAA GAA GGC CCC AAC ACG TTC CAG CTT TCT GAT CCA ATG CTC 441
    Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro Met Leu
    470

70  ACC ATG CTG GTC GAC AGT GCT TTA AAG GAT GAG CTT GTT TTG GGG GAT 489
    Thr Met Val Val Asp Ser Gly Leu Lys Asp Glu Leu Val Leu Gly Asp
    485
    
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	GAT	CCA	CCT	COG	TTT	CTG	TGG	AAC	AGC	AAG	TTG	AGC	CCG	GTG	537
Pro	AMP	Al	Pro	Arg	Phe	Val	Leu	Trp	Asn	Arg	Lys	Leu	Arg	Pro	Val
500					505				510					515	
5	CCC	GGG	AAG	CTG	ACT	GAT	TTG	CCT	TTT	GAC	TTG	ATG	AGC	ATT	GCT
Pro	Gly	Lys	Leu	Thr	Asp	Leu	Pro	Phe	Phe	Asp	Leu	Met	Ser	Ile	Gly
				520					525					530	
10	GGC	AAA	ATC	AGG	GCT	GCT	TTT	GCT	CCG	CTT	GAT	CGC	CCT	CCT	CCT
Gly	Lys	Ile	Arg	Ala	Gly	Phe	Gly	Ala	Leu	Gly	Ile	Arg	Pro	Pro	Pro
				535					540					545	
15	CCA	GCT	CAT	GAG	GAA	TGG	GTT	GAA	GAG	TTT	GTT	CGT	CGG	AAC	CTT
Pro	Gly	His	Glu	Glu	Ser	Val	Glu	Glu	Glu	Phe	Val	Arg	Asn	Leu	Gly
				550					555					560	
20	GAT	GAG	GTT	TTT	GAA	CCG	TGG	ATA	GAG	CCT	TTT	TGT	TCA	GGG	GTC
Arg	Glu	Val	Phe	Glu	Arg	Glu	Ile	Ile	Pro	Phe	Cys	Ser	Gly	Val	Tyr
				565					570					575	
25	GCA	GCC	GAT	CCT	TCA	AAA	TTA	AGT	ATG	AAA	GCA	TTC	GGG	AAA	GTT
Ala	Gly	Asp	Pro	Ser	Lys	Leu	Ser	Met	Lys	Ala	Ala	Phe	Gly	Lys	Val
				580					585					590	
30	TGG	AAG	CTG	GAA	AAA	AAT	GAT	GCT	AGC	ATT	ATT	GCT	GGA	ACT	TTT
Trp	Lys	Leu	Glu	Lys	Asn	Gly	Gly	Ser	Ile	Ile	Gly	Gly	Thr	Phe	Lys
				600					605					610	
35	GCA	ATA	CAA	GAG	AGA	AAT	GGA	CCT	TCA	AAA	CCA	CCT	GGA	GAT	CCG
Ala	Ile	Gln	Gln	Gly	Arg	Asn	Gly	Ala	Ser	Lys	Pro	Pro	Arg	Asp	Arg
				615					620					625	
40	CTG	CCA	AAA	CCA	AAA	GCT	CAG	ACT	GTT	GGA	TCT	TTT	CCG	AAG	GGA
Leu	Pro	Lys	Pro	Lys	Gly	Gln	Thr	Val	Gly	Ser	Phe	Arg	Lys	Gly	Leu
				630					635					640	
45	ACC	ATG	TTG	CCT	GAT	GCA	ATT	TCT	CCC	AGA	CTA	GGC	AAC	AAA	GTA
Thr	Met	Leu	Pro	Asp	Ala	Ile	Ser	Ala	Arg	Lys	Gly	Asn	Lys	Val	Lys
				645					650					655	
50	TTA	TCT	TGG	AAG	CTT	TCA	AGT	ATT	AGT	AAA	CTG	GAT	AGT	GGA	GAG
Leu	Ser	Trp	Lys	Leu	Ser	Ser	Ile	Ser	Lys	Lys	Leu	Asp	Ser	Gly	Glu
				660					665					670	
55	AGT	TTG	ACA	TAT	GAA	ACA	CCA	GAA	GGA	GTC	GTT	TCT	TTG	CAG	TGC
Ser	Leu	Thr	Tyr	Glu	Thr	Pro	Glu	Gly	Val	Val	Ser	Leu	Gln	Cys	Lys
				680					685					690	
60	ACT	GTT	GTC	ACC	ATT	CCT	TCC	TAT	GTT	GCT	AGT	ACA	TTG	CTG	CGT
Thr	Val	Val	Leu	Thr	Ile	Pro	Ser	Tyr	Val	Ala	Ser	Thr	Leu	Leu	Arg
				695					700					705	
65	CCT	CTG	TCT	GCT	GCT	GCT	GCA	GAT	GCA	CTT	TCA	AAG	TTT	TAT	TAC
Pro	Leu	Ser	Ala	Ala	Ala	Ala	Asp	Ala	Leu	Ser	Lys	Phe	Tyr	Tyr	Pro
				710					715					720	
70	CCA	GTT	GCT	GCA	GTT	TCC	ATA	TGC	TAT	CCA	AAA	GAA	GCT	ATT	AGA
Pro	Val	Ala	Ala	Val	Ser	Ile	Ser	Tyr	Pro	Lys	Glu	Ala	Ile	Arg	Ser
				725					730					735	
75	GAA	TGC	TTG	ATA	GAT	GCT	GAG	TTG	AAG	GGG	TTT	GCT	GAA	TTG	CAT
Glu	Cys	Leu	Ile	Asp	Gly	Glu	Leu	Lys	Gly	Phe	Gly	Gln	Leu	His	Pro

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7          745          750          755
CCT ACC CAA GCA GTG GAA ACA TTA GGA ACT ATA TAC AGC TCA TCA CTA 1305
Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu
5          760          765          770
TTC CCC AAC GCA GCA CCA CCT GGA AGG GTT CTA CTC TTG AAT TAC ATT 1353
Phe Pro Asn Arg Ala Pro Pro Gly Arg Val Leu Leu Leu Asn Tyr Ile
          775          780          785
10 GCA GCA GCA ACT AAT ACT GGA ATT TTA TCG AAG ACC GAC AGT GAA CTT 1401
Gly Gly Ala Thr Asn Thr Gly Ile Leu Ser Lys Thr Asp Ser Glu Leu
          790          795          800
15 GTG GAA ACA GTT GAT GCA GAT TTC AGG AAA ATC CTT ATA AAC CCA AAT 1449
Val Glu Thr Val Asp Arg Arg Leu Arg Lys Ile Leu Ile Asn Pro Asn
          805          810          815
20 CCC CAG GAT CCA TTT GTA GTG GGG GTG AGA CTG TCG CCT CAA GCT ATT 1497
Ala Gln Asp Pro Phe Val Val Gly Val Arg Leu Trp Pro Gln Ala Ile
          820          825          830
CCA CAC TTC TTA GTT GGC CAT CTT GAT CTT CTA GAT GTT GCT AAA CCT 1545
Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Asp Val Ala Lys Ala
          835          840          845
25 TCT ATC AGA AAT ACT GGG TTT GAA GGG CTC TTC CTT GCG GGT AAT TAT 1593
Ser Ile Arg Asn Thr Gly Phe Glu Gly Leu Phe Leu Gly Gly Asn Tyr
          850          855          860
30 GTG TCT GGT GTT GCC TTG GCA CGA TGC GTT GAG CGA GGC TAT GAG CTA 1641
Val Ser Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu Val
          865          870          875
35 GCA GCT GAA GTA AAC GAT TTT CTC ACA AAT AGA GTC TAC AAA 1681
Ala Ala Glu Val Asn Asp Phe Leu Thr Asn Arg Val Tyr Lys
          880          885          890
TAGTAGCAGT TTTTCTTTT GTGTGGGAT GGGTGATGG ACTCTGCTGT TCGATTGAT 1743
40 TATAATATG TGAAGTTTC TCAAAATTCG TCGATAGGTT TTGGCGCGCT TCTATTGCG 1803
ATAATGTAAA ATCTCTTTTA ACTTTGAAAA AAAAAAAAAA AAAA 1847

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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 543 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) SEQUENCE DESCRIPTION: SEQ ID 12:

```

Met Val Ser Val Phe Asn Glu Ile Leu Phe Pro Pro Asn Gln Thr Leu
1          5          10          15
60 Leu Arg Pro Ser Leu His Ser Pro Thr Ser Phe Phe Thr Ser Pro Thr
          20          25          30
Arg Lys Phe Pro Arg Ser Arg Pro Asn Pro Ile Leu Arg Cys Ser Ile
          47

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35 40 45

Ala Glu Glu Ser Thr Ala Ser Pro Pro Lys Thr Arg Asp Ser Ala Pro  
50 55 60

5 Val Asp Cys Val Val Val Gly Gly Val Ser Gly Leu Cys Ile Ala  
65 70 75 80

Gln Ala Leu Ala Thr Lys His Ala Asn Ala Asn Val Val Thr Glu  
85 90 95

10 Ala Arg Asp Arg Val Gly Gly Asn Ile Thr Thr Met Glu Arg Asp Gly  
100 105 110

15 Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro Met  
115 120 125

Leu Thr Met Val Val Asp Ser Gly Leu Lys Asp Glu Leu Val Leu Gly  
130 135 140

20 Asp Pro Asp Ala Pro Arg Phe Val Leu Trp Asn Arg Lys Leu Arg Pro  
145 150 155 160

Val Pro Gly Lys Leu Thr Asp Leu Pro Phe Phe Asp Leu Met Ser Ile  
165 170 175

25 Gly Gly Lys Ile Arg Ala Gly Phe Gly Ala Leu Gly Ile Arg Pro Pro  
180 185 190

30 Pro Pro Gly His Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn Leu  
195 200 205

Gly Asp Glu Val Phe Cys Arg Leu Ile Glu Pro Phe Cys Ser Gly Val  
210 215 220

35 Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly Lys  
225 230 235 240

Val Trp Lys Leu Glu Lys Asn Gly Gly Ser Ile Ile Gly Gly Thr Phe  
245 250 255

40 Lys Ala Ile Gln Glu Arg Asn Gly Ala Ser Lys Pro Pro Arg Asp Pro  
260 265 270

45 Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Gly Ser Phe Arg Lys Gly  
275 280 285

Leu Thr Met Leu Pro Asp Ala Ile Ser Ala Arg Leu Gly Asn Lys Val  
290 295 300

50 Lys Leu Ser Trp Lys Leu Ser Ser Ile Ser Lys Leu Asp Ser Gly Glu  
305 310 315 320

Thr Ser Leu Thr Tyr Glu Thr Pro Glu Gly Val Val Ser Leu Gln Cys  
325 330 335

Lys Thr Val Val Leu Thr Ile Pro Ser Tyr Val Ala Ser Thr Leu Leu  
340 345 350

60 Arg Pro Leu Ser Ala Ala Ala Asp Ala Leu Ser Lys Phe Tyr Tyr  
355 360 365

Pro Pro Val Ala Ala Val Ser Ile Ser Tyr Pro Lys Glu Ala Ile Arg  
48

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370          375          380
Ser Glu Cys Leu Ile Asp Gly Glu Leu Lys Gly Phe Gly Gln Leu His
385          390          395
5 Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser
405          410          415
Leu Phe Pro Asn Arg Ala Pro Pro Gly Arg Val Leu Leu Leu Asn Tyr
10 420          425          430
Ile Gly Gly Ala Thr Asn Thr Gly Ile Leu Ser Lys Thr Asp Ser Glu
435          440          445
15 Leu Val Glu Thr Val Asp Arg Asp Leu Arg Lys Ile Leu Ile Asn Pro
450          455          460
Asn Ala Gln Asp Pro Phe Val Val Gly Val Arg Leu Trp Pro Gln Ala
465          470          475
20 Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Asp Val Ala Lys
485          490          495
Ala Ser Ile Arg Asn Thr Gly Phe Gly Leu Phe Leu Gly Gly Asn
500          505          510
25 Tyr Val Ser Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu
515          520          525
30 Val Ala Ala Glu Val Asn Asp Phe Leu Thr Asn Arg Val Tyr Lys
530          535          540

(2) INFORMATION FOR SEQ ID NO:13:
35 (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 583 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear
40 (ii) MOLECULE TYPE: DNA (genomic)
    (iii) HYPOTHETICAL: NO
45 (ix) FEATURE:
    (A) NAME/KEY: promoter
    (B) LOCATION: 1..583
    (D) OTHER INFORMATION: /function= 'arabidopsis protox-1
50 promoter"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
55 GAATTCGGAT CGAATTATAT AATTATCATA AATTGAATA AGCATGTTGC CTATTATTAA 120
AGAGGTTTAA TAAAGTTTGC TAATAATGGA CTTTGACTTC AAACCTGAT CTGATGTAAT 120
TAATTAATAT TTACATCAAA ATTGCTGCAC TAATATTACC AAATTAATAT ACTAAATGAT 160
TAATTCGCAG AAAAAGACAT AATTCAGAAAT AAAGGCTCAT TATGATAAAC ACOTATTGAA 240
CTTGATAAAG CAAAGCAAAA ATAATGGGTT TCAAGGTTTG GATTATATAT GACAAAAAAA 300

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5.
   
 AAAAAAGTT TGGTATATA TCTATTGGC CTATAACCA GTTATACAA TTGGGCGTA 360
   
 ACTAAATAA TAAATAAAC GTAATGGTC TTTTATATT TGGGTCAAC CCAACTCTAA 420
   
 ACCCAACCA AAAAAAGT ATACGGTAG GTACACAGC TTATGGTGT TGTGATTGCA 480
   
 GGTGAATAT TCTGTGTC TCTGCTTC TCTGAGAA GATTACCAA TCTGAAAAA 540
   
 10. ACCAAGAGC TGACAAATC CGAATTCTC TCGATTTCG ATG 583

(2) INFORMATION FOR SEQ ID NO:14:

15. (i) SEQUENCE CHARACTERISTICS:
   
 (A) LENGTH: 1048 base pairs
   
 (B) TYPE: nucleic acid
   
 (C) STRANDEDNESS: single
   
 (D) TOPOLOGY: linear

20. (ii) MOLECULE TYPE: DNA (genomic)

25. (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

30. TCGATCTTC TAGGCTGATC CCAATCTTT CCTCGAAGC CCTGGGCGC TGTGGCCCTT 60
   
 GGAGCTGGTC GCTGAAGA GCTTGTCTT TGGCCGAGC ATTGTGAGT ATATTGTGAC 120
   
 CTCGAGACT GACTTCCTT GTGTCACCT TGAGTGAGT TATGAGTGA CCTGACGTC 180
   
 35. CTCAGATGA TTCTCTCTC GAAGCCCTG GTCATTTCG AGAATCTGA ATCTTATTC 240
   
 CTCTTTGCG GAAATCTCT CAGCTTGAT GTACTCATC ACTTCTGAA CGAGCTTCT 300
   
 40. CAGAGTTGT GGAGCTTTC TGGCGAATA TTGGCTGTA GGTCTGAGC GAAGACCTT 360
   
 GATCATGGCC TCAATGACAA TCTCATTGG CACGCTAGC GCTTGTGCC TCAATGCAA 420
   
 GAACCTCTT ACATATGCT GAAGTATTC TTCTGTATC TGTGTGATC GGAACAGAC 480
   
 45. CTGAGCTGT ACCGACTTC TTGAAAGCC TTGGAAGCTA GTAACCAACA TGTGCTTAA 540
   
 CTCTGCCAC GAGCTCATG TCCCTGGCG AAGAGAGAA TACCATGTT GGGCTACAT 600
   
 50. CCGACTGCC ATGACCAAG ACTTCGCCAT GACTACAGT TTGACCCAT ACAAGATAT 660
   
 ACTTCTTTC TAGCTCATCA GAACTCTTT TGGATCTGAG TCCCATCAT ACATGGGAG 720
   
 CTGAGCTGCG TTGTATGAT GGAACCATG GGTAGCTCG AGTTCTGCT GCAAGGAGA 780
   
 55. AGCATATCA AAGTAAAGC CATCATGAT AAAATCATCA TACCATCAT CCTGTTGAA 840
   
 TAACTCTCT TGACGAAGC CCTGTGTTG GGGCTTGA TCTTGTGAT CTTGAACAAG 900
   
 60. ATGACCACT TCTTCACTG CTTCGTGAT CTTCTTTTG AGATCAGCA GTCCACCAT 960
   
 CTCTCTCTC TTCTTTGTA CTGTGTTATG GATGATCCG ATGTCCTCA TCTCTTGGT 1020

	CAACTCTCC	TCTTGAGTG	TCAGACTGGT	GCTTTCCTC	TCCTGGCTTC	GAGCCCTCG	1080
	GAGAAAGA	GTTCCTGAT	TTGGGTCCAG	CGCTGCCAGT	GCAGTGGTCC	CTGGTGCTGA	1140
5	AGCTTTCTTC	GCTGCGATGA	CAAAAGTCCG	TGCTTGCCGA	AGGTGGTCCA	AAAGGGTTCA	1200
	CTAGAGGTGG	GAGCCAAATGT	TGGGGACTTC	TCAAGTCTTA	TGAGTTAAGA	ACAAGGCCAC	1260
10	ACAAAATGTT	AAAATTAAT	AGCTTTTCATC	TTTGGAAGCA	TTATTTCCCT	TTGGGTATAA	1320
	TGATCTTCAG	ACGAAAGAGT	CGTTCAATCA	TGGGATATAT	GTTAATGAA	GGAGGAGCAT	1380
	ATGAATGTA	AGAGCAACCA	TGAACATCG	TGTAGCATTG	TTAATTCATC	ATCATTTTAT	1440
15	TATTAATGAA	AAATGAAGAC	AATATTGAAT	TACAATGTA	CGCTTGGCTT	GACAGAAGAT	1500
	AAAAGTACAA	GCTTGAAGCA	CGAGCAAGTA	CAAGTCAGTG	TGAACAGTAC	GGGGTACTG	1560
20	TTCACTATAT	TATAGGCACA	GGACACAGCC	TGTGAGAAAT	TACAGTCATG	CGCTTTACAT	1620
	TTACTATTGA	CTTATAGAAA	ATCTATGAG	GACTGGATAG	CGTTTTCCCT	TTAAGTCCG	1680
	TGCTTTTTC	CGGATTAG	CGGAATCTCC	CTTGCCGATA	GCTTGGAGC	ATGGGCAAC	1740
25	TTCTTCACGA	TGATGCCCTT	CTCATTTGTT	ATGCTTTTAA	TGCTGAATTC	GAGGTACTCT	1800
	GTCCATAAAC	CATACCTTGA	AGACATGTTT	AAATTAATTT	TTTGAGGACT	TTGGGAGGAC	1860
30	GAAGGCCCTC	AACAGTCTGT	TTTTTGAGGA	CGTTGGAGG	ATGAAGGCCCT	CGAACAGGAC	1920
	CTATCCATAA	AACCAACCTA	TCCAGAAAC	CGAGCCCATC	CAGCTTCAT	TTGGCTTACC	1980
	AACACCCCTA	ATTAGCTGTT	TGCTTTAAAT	TTTTTAGGCT	CAATTTGGCT	ATCAGCATCC	2040
35	ACTGTCACTC	CACAAACTCA	TATCAATAA	ACAGACTCAA	TCAAGCCAAAC	TGACATATCC	2100
	CATAAAACCG	CGCCACCTCT	CTAGGCCCTC	GCCAGAAAC	AGAAACCGCT	ATTCAGAGTT	2160
40	CAACTTAA	AGGACATAA	CTTTCACCTT	GGAAGTGGAA	TGAGTGCAT	TTTTTTCCAA	2220
	ATCAGACAAA	ATTAAATTC	GCATCCGATA	ATCAAGCCAT	CTCTTCAGTA	TGTTTTTAAG	2280
	TGTTGCTCAC	ACTAGTGTAT	TTATGGACTA	ATCAGCTGTG	TATCTCATAC	ATAACATAT	2340
45	CGATACATCT	AGGTGTATC	TCAATTACCA	AAACCGAATT	ATAGCTTCC	AAAAAGGTTA	2400
	TGGACTAGTC	ACTCAATTAC	CAAAACTAAA	CTTTAGACTT	TGATGTATGA	CATCCACAT	2460
	GACACTGTAC	TGGACTAAAC	CAGCTTTCAA	GCTACACAAG	GAGCAAAAAT	AACTAATCT	2520
50	CGTATGTGTA	GGAGCTAAAG	TATATGTCCA	CAACAATAGT	TAGGGGAAGC	CGCCCAAGGAC	2580
	TTAAATTTCC	TTTTACCTCT	TGAAACTTTT	GTCTGTCTCT	ACTTTTTCAC	TTTAAACTTC	2640
55	AAAATTTGAC	ATTTTATCAC	CGCTTAACTC	TTAAACCAT	TTAAATTACA	TTCTTACTAG	2700
	ATTATAGATG	ATTTTGTGTT	GAAAAGTTTT	TAGACATGTT	TTACACATGG	ATTAAATACA	2760
60	TTTTTCAAT	TTCTTAGAGT	TAAATCTAA	CTTATTAAAA	CTATTATAGA	TACTTTCCAG	2820
	AGCTCTAAAT	ATTTTATTTT	TTTCATTATG	GAATTTGTTT	AGAACTCTTA	TAGACCTTTT	2880
	TTTTTGTTTT	AAAAGCTTGG	CGATGTTTTT	AAACAATTTT	TTTTCTATTT	TTTGAATTTT	2940

	TTGGAAG CACTTCTAC CGGTAGAG ATTATTTC CTACATTAT ATCTAGACA	3000
	AAATCACTT ATGAAATGT CTGGAAGT ACCTCTAAC CGGTAGATG AATTGAAATG	3060
5	AAAATTAAAC CAACTTACGG AATCGCCCA CATATGTGA TTAAGTGA TATGATACA	3120
	TATGAAGAG CCTAGAGAT AATCTAAATG GTTTCAGAT TGAAGTTAT TTTTGAAGT	3180
10	TTGATGGAA GATAAGACA TAACTGTAGT TCACAGAGT AAAAGGTTA TTTTTCAG	3240
	AAATATTGT GCTGCAATG ATCTGTGCC TCAAAATCAG CCTGCACTA AGCCAGGTT	3300
	CTAGACGAA CAGGCCAC GTACCCCTG GCCCTCAGG CGAAGCAGT CTGTGTCAGA	3360
15	CTTGAGAGG GATTGATAT CAACGGAAC AATCAAGCA GGCATTCGA TTCCAGGCC	3420
	ACCTGTAACT TTGAGTGGG CCACTCTTAA CTCCAGGCC AACGCCCTA CCCCATCTG	3480
20	TCTGTCTAT CACTGCGCG CACAGCGCT CAGCTCCCA AGCCGCGCG AAATGCTGC	3540
	CGCCACAGC ACCGCCATG CGACGCTGC ATGCCGCTA CTCACGGGA CCGGAATACC	3600
	TCCGCTCTC CGCCATCGG GACTCAGGT GCGCTGGCT GCTGTGGGG GCGCGCGCG	3660
25	CGAGGCACC GCATCCACG GCGCGCGCT GTCCGCGAC TCGTTGTGT TCGCGGAGG	3720
	CATCATGCG CTCTGACCG CGCAGCGCT GCGCAGCGG CAGCGCTGT GCGAGCTGT	3780
30	TGTCAAGAG GCCCGCGCC GCGCGCGCG CAACATTACC ACCGTGAGC GCGCGAGGA	3840
	ACGGTACC	3848

The invention as described herein is contemplated to include the following enumerated embodiments:

- 5 1. A recombinant DNA molecule comprising a plant protoporphyrinogen oxidase (protox) promoter or a functionally equivalent derivative thereof.
2. A chimeric gene comprising a plant protox promoter operably linked to a heterologous DNA coding sequence.
- 10 3. The chimeric gene of claim 2 wherein said plant protox promoter is from a protox-1 gene.
4. The chimeric gene of claim 2 wherein said plant protox promoter is from a protox-2
- 15 gene.
5. The chimeric gene of claim 2 wherein said protox promoter is from a plant selected from the group consisting of *Arabidopsis*, soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grass and rice.
- 20 6. The chimeric gene of claim 5 wherein said promoter is from a plant selected from the group consisting of *Arabidopsis* and maize.
7. The chimeric gene of claim 6 wherein said promoter is at least 300 nucleotides in
- 25 length.
8. The chimeric gene of claim 7 wherein said promoter is at least 500 nucleotides in length.



9. The chimeric gene of claim 8 wherein said promoter is from *Arabidopsis* and has the sequence set forth in SEQ ID No. 13.

10. The chimeric gene of claim 8 wherein said promoter is from maize and has the sequence set forth in SEQ ID No. 14.

11. The chimeric gene of claim 2 wherein said heterologous coding sequence encodes a modified, herbicide-resistant form of a plant enzyme.

12. The chimeric gene of claim 11 wherein said plant enzyme is selected from the group consisting of imidazoleglycerol phosphate dehydratase (IGPD), EPSP synthase, glutamine synthetase (GS), acetyl coenzyme A carboxylase, acetolactate synthase, and protoporphyrinogen oxidase (protox).

13. The chimeric gene of claim 12 wherein said plant enzyme is protox.

14. A recombinant DNA vector comprising the recombinant DNA molecule of claim 1.

15. Plant tissue comprising the chimeric gene of claim 2.

16. A plant comprising the chimeric gene of claim 2.

17. The plant of claim 16 wherein said plant is selected from the group consisting of *Arabidopsis*, soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grass and rice.

ABSTRACT OF DISCLOSURE

- 5 Promoters naturally associated with plant protoporphyrinogen oxidase (*protox*) coding sequences, and derivatives thereof, are provided. These promoters can be used to control the expression of an operably linked heterologous coding sequence in a plant cell. These promoters are particularly useful for expressing modified forms of herbicide target enzymes, particularly modified forms of *protox*, to achieve tolerance to herbicides which inhibit the corresponding
- 10 unmodified enzymes. Recombinant DNA molecules and chimeric genes comprising these promoters are provided, as well as plant tissue and plants containing such chimeric genes.